HEMATOLOGICAL MARKERS OF RADIATION INJURY

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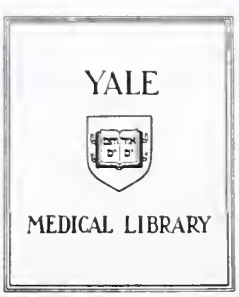

HEMATOLOGICAL MARKERS OF RADIATION INJURY

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I would like to thank Dr. Stuart Finch who was the advisor for this thesis. It was as a freshman medical student that I first encountered his enthusiasm for Japan and, through his continuing encouragement, it subsequently became possible for me to journey to Hiroshima to work with him. He was a great teacher and a warm friend. His humility would eclipse any effort of mine to thank him properly.

I would also like to thank Drs. James Norman and Kelvin Lee, both of the Department of Statistics at the Radiation Effects Research Foundation, who provided astute assistance in the retrieval and analysis of my data. Mr. Geoff Day, a kindly Australian who has worked in Hiroshima for over thirty years, lent me sage advice and unending technical assistance - I owe him many thanks.

Finally, I would like to acknowledge and thank Dr. Rose Papac of the Department of Medicine, Yale Medical School, who graciously agreed to read this thesis in its earliest form and provide editorial comment.

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PREFACE

In the early morning of August 6, 1945, the American B-29 bomber "Enola Gay" lifted off the runway in the Marianas and motored westward over the Pacific, toward the archipelago of Japan. Its destination was the city of Hiroshima, situated in western Honshu. At 8:15 am a 12.5 kiloton atomic bomb was let down by parachute and detonated, 576 meters above the city. Below, it left a circular wasteland of life and form. An area 13.2 km^2 was leveled. Loss of civilian life was numbered at 78,150. More than half that number were either injured or missing. The casualties sustained by the military personnel have never been reported. Within three days the city of Nagasaki, nestled at the edge of the southern island of Kyushu, became the second and hopefully last city in the world to fall witness to the cataclysmic capacity of nuclear warfare. On August 9th, a 22 Kiloton plutonium bomb was exploded 507 meters above the harbor city, taking the lives of 23,753 people. Another 23,345 were left injured with 1,924 missing in the rubble.

And so the war ended, the Occupation forces arrived and Japan miraculously pulled itself back up from the dust; but Hiroshima and Nagasaki continued to limp - crippled by radiation effects. Most of these effects were biological realities. Some were unsubstantiated fears fostered by the imagination of anxious survivors. Radiation injury was a disease fashioned by man, but poorly understood by him. The entire world was watching Hiroshima and Nagasaki and in the

ensuing thirty years we learned some grim lessons through the study of radiation biology in these two cities.

This thesis was written in Hiroshima during the early part of 1976. The majority of data was gleaned in a rather dispassionate manner from magnetic tape files and coverslip slides. Please remember that these lifeless numbers come from living people- the many survivors of Hiroshima and Nagasaki who count themselves among the millions of innocent people victimized by wars they barely understood. It would be a sacrilege to launch into a dissertation on radiation effects without first giving praise to the people of both these cities who have so willingly and graciously cooperated in their followup care.

THE RADIATION EFFECTS RESEARCH FOUNDATION

The work for this thesis was carried out at the Radiation Effects Research Foundation (RERF) in Hiroshima, Japan. RERF was formerly known as the Atomic Bomb Casulaty Commission (ABCC), a research organization established in 1947 by a directive of the President of the United States. It emerged under the guidance of the National Academy of Sciences-National Research Council - its extended mission was to monitor the health of the survivors of the atomic bombs.. It was hoped that, through the study of this population, we might document and perhaps come to understand and control the damaging effects of radiation. This knowledge would be essential as the world began to realize the peaceful potential of atomic energy.

Initially, ABCC was funded by the Atomic Energy Commission with additional monies in later years coming from the U.S. Public Health Service, the National Cancer Institute, and the National Institute of Heart and Lung Diseases. In 1948 the Japanese became co-participants in the studies through the cooperation of the Japanese National Institute of Health.

In April, 1975, after twenty-eight years of operation, ABCC was superseded by the Radiation Effects Research Foundation which is organized as a private Japanese foundation, sponsored by the Japan Ministry of Health and Welfare and the foreign Ministry. It is funded equally by the governments of Japan and the United

States. American support is provided through the National Academy of Sciences under contracts with the Energy Research and Development Administration (ERDA), the National Cancer Institute and the National Institute of Heart and Lung Diseases. In the fashion established by ABCC, RERF has laboratory and examination facilities in both Hiroshima and Nagasaki. There is an ongoing research effort in both cities with concerted study of radiation effects at many different levels, from systematic biennial clinical evaluations of an adult population to meticulous biochemical genetic studies on the f_1 generation of survivors. An excellent review of the major findings of the Atomic Bomb Casualty Commission has been recently published (1).

INTRODUCTION

The acute, chronic, and latent effects of exposure to ionizing radiation are protean. They have been extensively researched and recorded in both human and animal models. At the Atomic Bomb Casualty Commission, over thirty years of research was completed and a large library of technical reports has been compiled. Most of these reports have subsequently appeared in the medical literature throughout the world. Aside from the acute effects of exposure to radiation there are many persistent effects, most of which have been elucidated from a systematic evaluation of the large population of survivors in both Hiroshima and Nagasaki. Statistical studies have documented an increased incidence of leukemia (2-4) and thyroid carcinoma (5) as well as a probable increase in the occurrence of other solid tumors (6) in the more heavily irradiated survivors (in particular lung and breast carcinoma (7-11)). Radiation "markers" isolated at ABCC include chromosomal aberrations in circulating leukocytes (12-13), radiation cataracts (posterior lenticular opacities) (14), microcephaly, and mental retardation amongst those exposed in-utero (15) as well as a slight dimunition in growth and development among children exposed at a very young age. One of the most significant and encouraging findings to date involves the evaluation of over 70,000 f_1 children which failed to demonstrate any clinical evidence of a radiation effect (16).

This thesis was not conceived as a treatise on radiation

biology nor as an encompassing review of "radiation effects". It was begun with the idea that by evaluating the extent of radiation damage in a selected organ system one might be able to approximate the dose of ionizing radiation that that particular organ system had received, i.e. to use the magnitude of specific organ damage as a biologic dosimeter. The majority of persons exposed during the atomic bombings have had so called T65D doses calculated for them. (A discussion of T65D doses can be found in the 2nd section of part I). There remains, however, a substantial population of survivors of whom T65D could not be calculated. This group of survivors is usually included in the "unknown" dose group in any major population study at RERF and the uncertainty of their extent of exposure has hampered many studies. This has been particularly true when investigating the incidence of low frequency diseases (such as breast cancer) where the absolute number of cases is small and the presence of several "unknown" patients has a marked effect on the statistical significance of the results. If by evaluating a potential radiation marker (biologic dosimeter) one might give a rough dose estimate to a subject with previously unknown dose, these "unknown" dose individuals might then be included in the numerous statistical studies that are being conducted at RERF. It should be emphasized that the potential for biologic dosimeters extends beyond the facilitation of statistical analysis in Hiroshima and Nagasaki. In the event of any nuclear accident or catastrophe, the evaluation of biological aberrations would have both diagnostic and prognostic importance.

THESIS:

This thesis will describe an evaluation of gradients between dose, and the frequency of abnormalities, in two potential "late" radiation markers- bone marrow cytology and absolute lymphocyte counts. Both investigations were carried out on selected patients with established exposure doses. The two systems- bone marrow cytology and absolute lymphocyte counts were analyzed in an attempt to establish biologic dosimeters which would, in turn, facilitate the estimation of exposure doses for unknown survivors.

The thesis is divided into two independent sections.

I. BONE MARROW CYTOLOGY - 1950 - Five years post-exposure to whole body irradiation. Hiroshima, Japan.

II. THE EFFECTS OF RADIATION ON ABSOLUTE LYMPHOCYTE COUNTS IN THE ADULT HEALTH STUDY SAMPLE HIROSHIMA-NAGASAKI, JAPAN.

PART I. BONE MARROW CYTOLOGY - 1950 - Five years post exposure to whole-body irradiation. Hiroshima, Japan.

Section 1. Introduction

A study was undertaken to evaluate the "late" (five year) effects of mixed gamma-neutron irradiation on the bone marrow cytology of a group of survivors of the atomic bomb in Hiroshima.

The acute effects of radiation on hematopoietic tissue have been well documented. Investigations in Hiroshima and Nagasaki in addition to experiences with accidental fallout (7) nuclear reactor and accelerator accidents (18-19) therapeutic and diagnostic radiology (20) as well as experimental animal studies (21) have provided a vast amount of data on the early perturbations of hematologic parameters immediately following whole body irradiation.

Blaisdell has reviewed the ABCC hematologic studies that were carried out in Hiroshima and Nagasaki during the years 1947-59 (22). The earliest hematologic findings from these two cities were reported by Le Roy in 1950 (23-24). The data in his report includes both the evaluations by the Joint Commission for the Investigation of the Atomic Bomb (which functioned from the 26th September - 16th December, 1945) as well as the initial studies of Japanese physicians and field workers who performed blood examinations prior to the formation of the Joint Commission. These early reports were recorded under compromised conditions. A systematic, well controlled examination could not have been performed at that time.

Little is known about the acute changes in the bone

marrow of Hiroshima survivors as few aspirates were obtained in the first days following the atomic blast. Many examinations were performed later, at four to six weeks, and revealed hypoplastic marrows with a return to what was considered a normal pattern of myelopoiesis and megakaryopoiesis at approximately seven to eight weeks- post irradiation. Erythropoiesis was found to lag, returning to normal around the tenth to twelfth week. By the sixteenth week, bone marrow samples from the survivors were described as completely normal (22).

Many "late" (non acute) bone marrow examinations were performed at ABCC in the years following its inception in 1947. A statistical evaluation of this data has unequivocally documented a dramatic increase in the incidence of leukemia (all series except chronic lymphocytic) amongst the survivors of Hiroshima and Nagasaki during the early years following the blast. More recently, the incidence of hematologic malignancies still remains moderately elevated (2,7,25). Thousands of bone marrow aspirates were obtained at ABCC as part of the clinical investigation of suspected leukemic or pre-leukemic states, as well as refractory anemias and thrombocytopenias. There is no record of "late" bone marrow samples from non-leukemic survivors having been studied for persistent cytologic abnormalities.

THESIS:

To review a set of bone marrow aspirates obtained from

Hiroshima survivors in 1950, five years after exposure, in an attempt to isolate and describe cytologic abnormalities. In addition, to analyze the frequency of abnormalities as a function of exposure dosage looking for a possible gradient.

Section 2

MATERIALS:

Slides were recently recovered from fifty-four bone marrow aspirations performed on a group of heavily exposed Hiroshima survivors who were part of a radiation cataract study. These aspirates were obtained in 1950, five years following exposure to mixed gamma-neutron radiation from the Uranium^{235} atomic bomb explosion in Hiroshima. These marrow aspirates were part of an extensive clinical and laboratory investigation that was carried out on seventy-eight survivors with documented radiation cataracts (posterior lenticular opacities). The purpose of the study was to determine if other late manifestations of radiation injury existed in this population with known radiation induced ophthalmological disease. The initial findings from this study were reported by Fillmore (26) . No other disease attributable to the atomic bomb was detected. Fillmore's only reference to the bone marrow samples obtained was that.. "none gave evidence of serious disease." No other review of this set of bone marrows was subsequently undertaken.

Of the 54 patients for whom bone marrow slides were available, T65D doses* were calculated for 45. The distribution of subjects with different doses is seen in Table below.

TABLE I

Number of subjects	T65D doses (rad)
3	greater than 1000 r
4	751-1000 r
10	501-750 r
9	401-500
9	301-400
8	201-300
1	101-200
1	less than 100 r
9	not calculated

The distribution included 30 males and 24 females with ages between 12 - 69 years. Control specimens were not drawn at the time of

*T65D doses: In the late 60's and early 70's T65D dose became available for the majority of survivors in Hiroshima and Nagasaki. T65D refers to a particular dosimetry system which was first proposed in 1965 by collaborators at Oak Ridge National Laboratory and the Japanese National Institute of Radiological Sciences. Each dose estimate is calculated from data derived from detailed histories which extracted information regarding location and shielding at the time of the blast. The dose estimate can be separated into neutron and gamma contributions and is believed to be accurate to within approximately 30% for individual survivors (27-28). It was not possible to calculate T65D doses in some cases. These included people whose shielding configuration at the time of the blast was too complex for dose estimation (e.g. exposure in a crowded street-car). There was also a sizable number of people, including small children, who were unable to remember the details of their location at the time of the blast. (Continued on next page)

the ophthalmology study in 1950. A search of the RERF bone marrow files was made in an attempt to locate suitable controls. Only five samples were felt to be adequate. None of the controls were in the city at the time of the blast. See table II.

TABLE II. Controls:

AGE	SEX	YEAR	DIAGNOSIS
48	F	1963	menorrhagia
2	M	1952	hepatomegaly
62	M	1958	Laennec's cirrhosis
62	F	1969	hemosiderosis
75	M	1957	pernicious anemia in remission

Section 3

METHODS:

Specimens without T65D dose assignment were deleted from the study. Also deleted were nine marrows which were felt to be of too poor quality to be adequately reviewed. The five controls were added to the remaining thirty-five marrows from the ophthalmology study, and each marrow was then reviewed blindly without knowledge of exposure dosage. When possible, 4000 erythroblasts (including proerythroblasts, early and late basophilic normoblasts, polychromatophilic and oxyphilic normoblasts) were counted for each

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T65D is not a whole body dose but represents the first collision absorbed dose to a small mass of tissue in the radiation field, where the survivor was located at the time of exposure. The total dose is computed from the sum of gamma and first collision neutron doses in rad. (1 rad = 100 ergs/g=10^{-2}J/kg). In the near future dosimetry calculations will permit specific tissue dose estimates e.g. bone marrow doses.

patient. In the course of counting 4000 erythroblasts, cytologic abnormalities, as they occured, were recorded. Particular attention was paid to abnormalities which have been associated with radiation injury in previously reported studies (29). These included bi- and trinucleated cells, chromosomal bridging in ana- and telephase, nuclear fragments (karyomeres), abnormal mitoses, hypersegmentation and giant nuclei. Myeloid precursors were not enumerated, though aberrations in this cell line were noted in relation to the 4000 erythroblasts counted. Histologic evaluation of a selection of bone marrow smears was performed without knowledge of radiation dose by an additional experienced hematologist* for purpose of verification.

The clinical charts for the exposed individuals were available for review. Pertinent information obtained from the charts included the following clinical data; recorded at the time of marrow aspiration in 1950.

a. Hematologic parameters (RBC, HGB, HCT, indices, reticulocyte count, WBC and differential count)
b. Clinical state
c. Parasitology (stool)
d. Mycology (sputum for AFB)
e. Serology

The subsequent clinical course over the following years was also noted.

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* Dr. Stuart C. Finch, Chief of Research at RERF

Section 4

RESULTS:

A summary of findings for each bone marrow aspirate reviewed can be found in Table III. The most frequent abnormality noted was binucleation of cells in the erythrocytic series. Binucleated erythroblasts were divided into "early" and "late" according to the maturation characteristics of their nuclei and cytoplasm. Figures 1-4 and 5-6 give examples of both. "Early" erythroblasts included pronormoblasts and basophilic normoblasts. The transition between basophilic normoblast and polychromatophilic normoblasts provided a vague dividing line between "early" and "late" erythroblasts. Any cell that was clearly identified as a polychromatophilic normoblast or orthochromatic normoblast was included in the "late" binucleated series.

Some uncertainty arose when attempting to differentiate a true binucleated cell from a mitotic cell, late in anaphase, prior to initiation of cytokinesis. In this study, a cell was considered to be a "late" mitotic cell if the cytoplasm was overtly elliptoid with extreme eccentricity of the nuclei, Figures 7-8, or if the nuclear chromatin was not uniformly condensed, Figures 9-10.

In comparing the frequency of binucleated cells (either "early" or "late" erythroblasts) per 1000 erythroblasts, no statistically significant difference between different dose groups and the controls was demonstrated (Table IV). Late mitoses, as defined

above, were also enumerated. Statistical analysis failed to reveal a gradient with dose.

The frequency of karyomeres (Table V) was found to be independent of dose although the 600+ rad group had a striking incidence of 8 per 1000 erythroblasts. Examples of Karyomeres can be seen in figures 10-14.

Mitotic bridging ("bridging" of chromosomal material between segregating fragments (figures 15-19) was noted only in the exposed samples though a gradient with dose was not demonstrated (Table V). Because the number of controls is small, the absence of bridging within this group is not statistically significant.

Internuclear bridges between interphase erythroid cells, (figures 20 - 25), were noted in seven marrows. All but one (#52- dose 140 r) had and exposure of greater that 300 rad. Strict criteria were used for identifying internuclear bridges. Many examples of intercytoplasmic bridging were noted but only those cells which were clearly linked from nucleus to nucleus with a fine strand of chromatin like material were counted. Though internuclear bridging was not observed in controls, their paucity precluded establishment of any statistical significance.

Mitotic figures were enumerated in the course of counting 4000 erythroblasts. When possible, they were differentiated as myelocytic or erythrocytic. Most were undetermined. A very

rough mitotic index (total number of mitoses, erythroid, myeloid, and undetermined combined, per 1000 erythroblasts) is reported in Table VI. There does appear to be a gradient with dose. This finding is suggestive but not significant ($.05<\rho<.10$).

Other rare findings in selected marrows included tri and quadranucleated erythroid and myeloid cells (figures 26-29) as well as binucleated plasma cells (figures 30-31), hypersegmentation, (figure 32) (only one case), and several instances of apparent multipolar mitoses. (figures 33-36). Though none of these abnormalities were noted in the controls, statistical significance cannot be attached.

There was excellent verification of the described bone marrow findings by the other hematologist, who examined selected marrow smears. There also was essential agreement between the examiners regarding the interpretation of several hundred photomicrographs of various cytologic aberrations which were observed by the primary examiner.

Medical records for thirty-two of the thirty-six exposed patients were available for review. Analysis of hematologic parameters at the time of bone marrow aspiration did not reveal anemia (all hemoglobin values greater than ten grams percent) or reticulocytosis (all reticulocyte counts less than 1.3 percent) in any of the cases. There were 17 cases of intestinal parasitism which were demonstrated in stool specimens obtained at the initial

clinic examination in 1950. This parasitism was emphasized by the absolute eosinophilia noted at the same time. Common parasites included Ascaris lumbricodese, hookworm, and T. trichiurus. It should be emphasized that despite the frequency of parasitism, anemia was not present in any of these cases. The remaining examination in 1950 included a complete physical exam, serology, urinalysis, and chest roentgenograms. No active disease process other that posterior lenticular degeneration was detected, excepting two cases of pulmonary tuberculosis

The clinical course subsequent to 1950 could be followed for a majority of the exposed individuals. Of eleven deaths noted in the ABCC files, the average age at death was 75. One subject (#40) died in 1962 at the age of 40 of gastric carcinoma. There were two other deaths secondary to gastric carcinoma (#2, #42). The remaining causes of death had no apparent relation to previous radiation exposure.*

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*An increased incidence of gastric carcinoma has not been linked to radiation exposure. These three cases of gastric carcinoma were isolated from the other eight deaths since they are cancers, and radiation is known to be carcinogenic.

TABLE III

CYTOLOGY OF INDIVIDUAL BONE MARROWS

SPECIAL BONE MARROW DIFFERENTIAL STUDIES, FIVE YEARS FOLLOWING EXPOSURE, HIROSHIMA

Case No.	M.F. No.	Dose in Rad	Erythroblasts Counted	Binucleated Cells			Late Mitosis		Tri/Quad Nucleated	Karyomeres		Internuclear Bridge	Mitotic Bridge	Mitosis			
				Early Erythrocytes	Late Erythrocytes	Myelocytes	Erythrocytes	Myelocytes		Erythrocytes	Myelocytes			Erythrocytes	Myelocytes	Undetermined	Total
1	216 373	440	4000	9	13	2	14		1 myelo	3	4		4	7	8	19	34
2	241 305	212	650	-	2						1			3	2		5
5	257 711	755	4000	1	5	1	4						1	7	8	34	49
6	400 428	443	4000	2	12	2	14			3	2		4	2	3	45	50
7	238 499	707	4000	5	11	3	14			4	1		2	2	14	42	58
8	242 288	348	4000	10	14	1	10	2	1 tri 1quad		1		8				46
9	277 937	313	4000	4	4	2	20			19	2	5	3	2	2	21	25
10	234 111	540	4000	3	4		10			2			2	1	1	35	37
14	236 627	567	3000	-	3	1	1			3	2			1	3	4	8
15	320 482	380	4000	4	13	1	7			2	3		3	4	9		37
16	215 018	997	4000	2	5	1	1			2			3	13	7	32	52
17	301 208	432	3000	1	15	1	3			2		1	1	2	2	24	28
19	256 830	1041	4000	2	3		5			2			1				27
20	299 397	269	4000	-	6	1	6			3			1		1	23	21
22	298 391	511	4000	1	7	-	7			3			2	4	4	18	26
23	242 305	382	1750	1	6	3	3			3			1	0	2	14	16
25	242 574	NIC	4000	2	10	1	5			3	1			1	4	39	44
26	299 233	228	4000	4	14	2	2		1 quad	8	2		4	1	5	49	55
27	241 276	322	4000	2	7	1	3			2	1		1		5	19	21
28	259 746	382	4000	2	13	1	3			7	2	1	1				39

Bone marrows not looked at: 3, 4, 12, 13, 18, 24, 29, 35, 51

TABLE III continued

Case No.	M.F. No.	Dose in Rad	Erythroblasts Counted	Binucleated Cells			Late Mitosis		Tri/Quad Nucleated	Karyomeres		Internuclear Bridge	Mitotic Bridge	Mitosis			
				Early Erythrocytes	Late Erythrocytes	Myelocytes	Erythrocytes	Myelocytes		Erythrocytes	Myelocytes			Erythrocytes	Myelocytes	Undetermined	Total
30	240 781	NIC	3000	2	4	9	1			3	1			1	3	12	16
31	400 896	711	4000	1	13	1	10		1 quad 1 tri	3			5	1	8	35	44
32	252 508	478	4000	2	10	-	1	1		1					8	29	37
33	268 304	293	4000	-	5	-	4		1 tri					1			20
36	306 758	96	4000	2	15	1	3			4			1	4	5	14	23
37	244 911	552	4000	-	7	2	5			1			2	1	3	17	21
40	282 568	652	4000	2	11	-	4	2	1 tri myelo	-		2		4	3	16	23
42	244 512	206	2000	-	5	1	4			-			1				17
45	323 591	280	3500	-	15	3	1			3					2	28	30
46	269 530	401	4000	-	10	2	8					3		4	8	6	18
47	314 612	NIC	4000	-	10	-	7			13							18
48	400 939	1568	3000	-	10	1	-			2							18
49	294 619	625	1000	1	4	-	5			10					2	11	13
50	400 932	434	4000	-	2	3	1		1 quad (?)								15
52	242 896	140	4000	4	13	-	2			5		2	1		4	37	41
53	474 938	NIC	1700	1	2	-	4										13
54	255 088	336	4000	1	10	3	3			7				7	11	20	38
55	478 435	NIC	4000	-	4	-	9			1							8
56	282 584	474	4000	2	6	-	3			1							34
57	300 792	317	4000	2	10	2	6			2		1					31
59	256 178	203	4000	-	11	2	5			7							18

TABLE IV

MITOTICALLY CONNECTED ABNORMALITIES

A. Binucleated and "late" mitotic cells

DOSE GROUP (rad)	n	BINUCLEATED CELLS "EARLY"	BINUCLEATED CELLS "LATE"	TOTAL BINUCLEATED	LATE MITOTIC CELLS	TOTAL BINUCLEATED + LATE MITOTIC
0/NIC	5	0.35 (0.15)	1.70 (0.33)	2.05 (0.34)	1.59 (0.37)	3.64 (0.38)
1-199	2	0.75 (0.13)	3.50 (0.25)	4.25 (-)	0.62 (0.13)	4.88 (0.13)
200-399	15	0.53 (0.18)	2.62 (0.24)	3.16 (0.31)	1.46 (0.32)	4.61 (0.43)
400-599	11	0.44 (0.17)	2.10 (0.37)	2.54 (0.44)	1.54 (0.36)	4.08 (0.68)
600+	8	0.49 (0.12)	2.42 (0.42)	2.90 (0.46)	1.61 (0.56)	4.51 (0.94)
DIFFERENCE IN MEANS		NS	NS	NS	NS	NS

n: number of marrows; NS: not significant (p .05)
Standard error in parenthesis.

TABLE V

MITOTICALLY CONNECTED ABNORMALITIES

B. Karyomeres, Mitotic Bridges

	DOSE GROUP (rad)					
	0/NIC	1-199	200-399	400-599	600+	DIFFERENCE IN MEANS
n	5	2	15	11	8	
KARYOMERES	1.05 (0.58)	1.13 (0.13)	1.13 (0.32)	0.46 (0.10)	8.00* (1.20)	NS
MITOTIC BRIDGES	0 -	0.25 -	0.38 (0.11)	0.33 (0.11)	0.36 (0.16)	NS

*One case had 10 karyomeres; std.error in parentheses

TABLE VI

ROUGH MITOTIC INDEX *

	0/NIC	1-199	200-399	400-599	600+	DIFFERENCE IN MEANS
n	5	2	15	11	8	
TOTAL MITOSES per 1000 erythroblasts	6.10 (1.52)	8.00 (2.25)	7.92 (0.62)	7.38 (0.92)	10.00 (1.15)	suggestive (.05 p .10)

*Includes mitoses in both erythroid and myeloid lines.

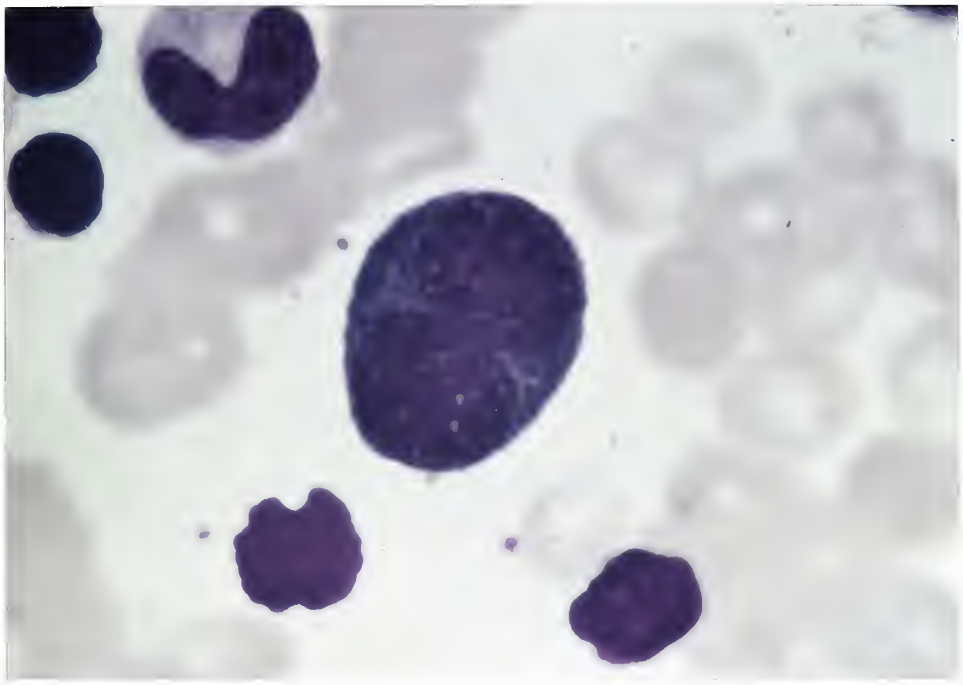

Figure 1. "Early" binucleated erythroblast

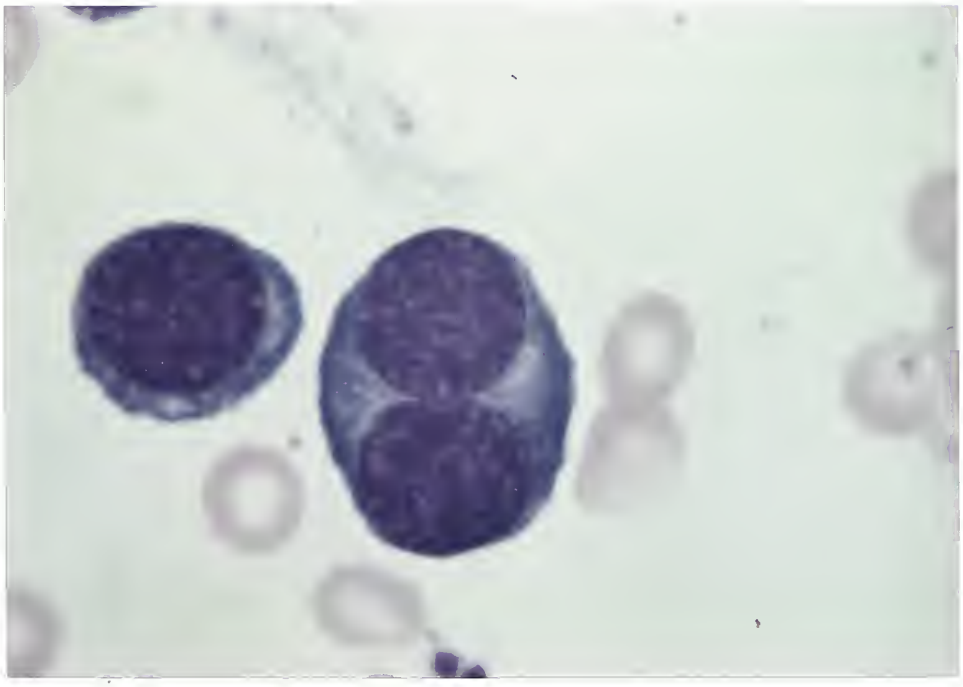

Figure 2. "Early" binucleated erythroblast

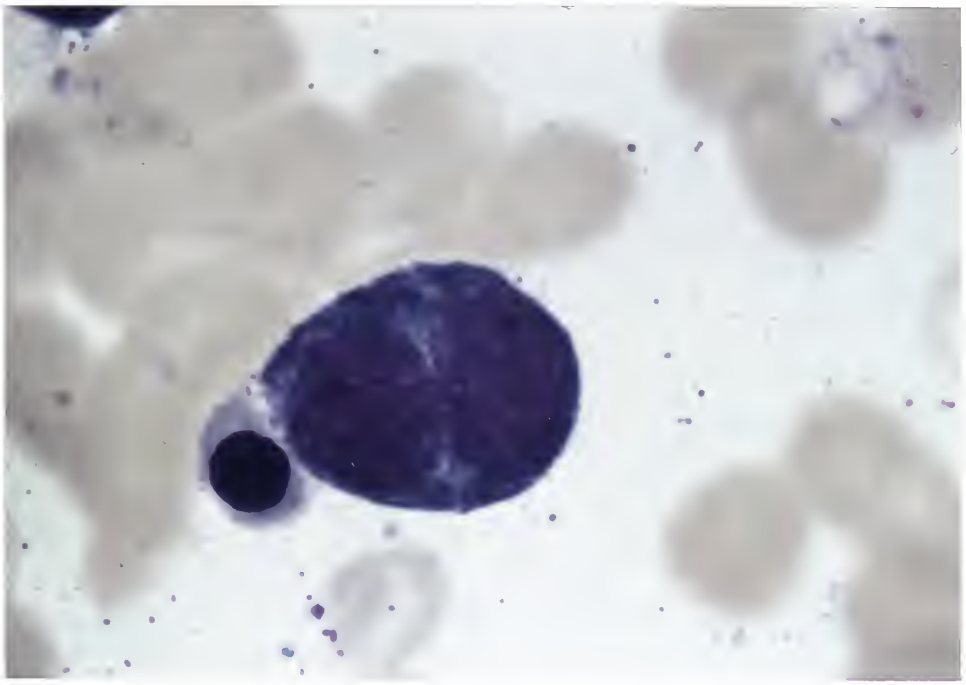

Figure 3. "Early" binucleated erythroblast

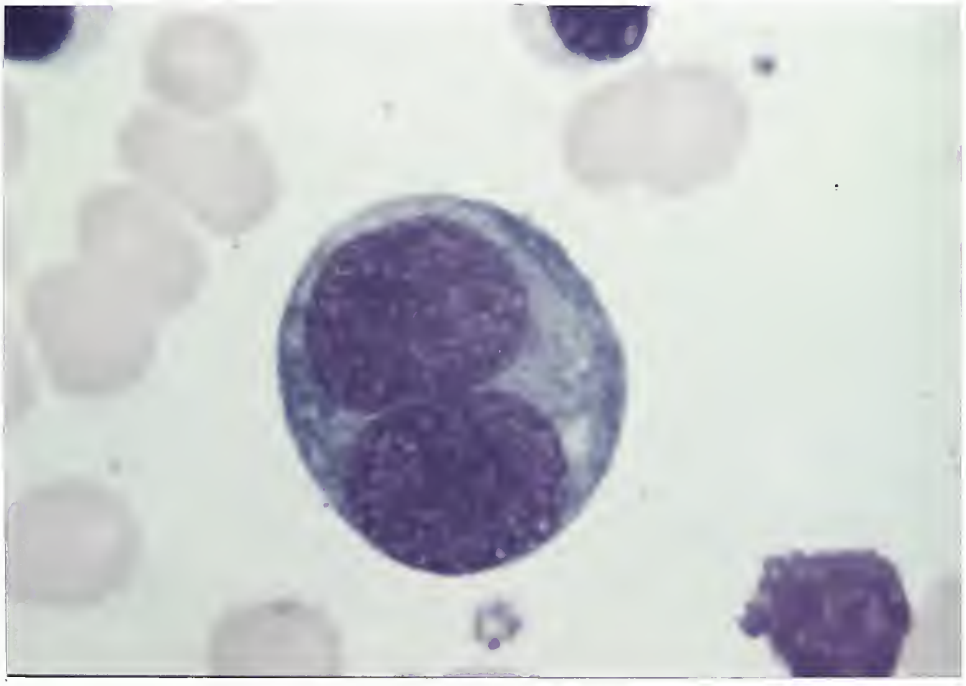

Figure 4. "Early" binucleated erythroblast

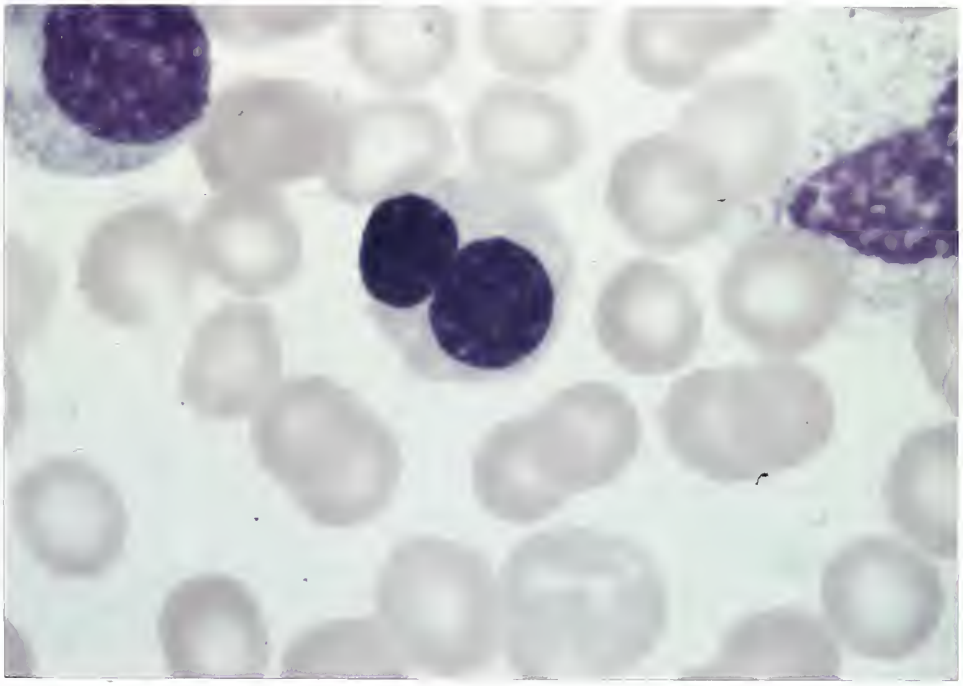

Figure 5. "Late" binucleated erythroblast

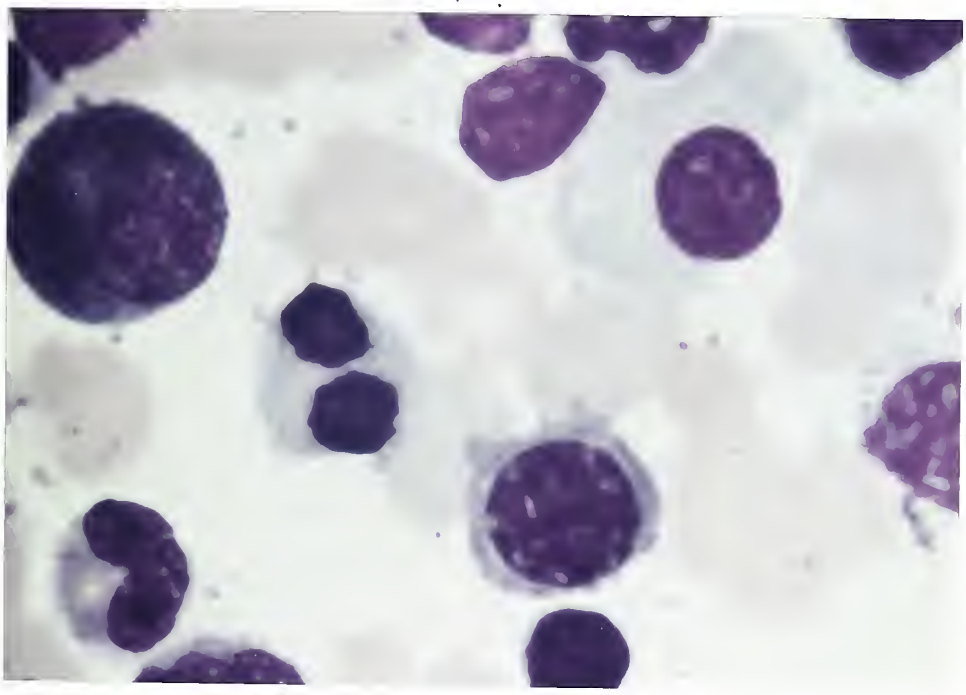

Figure 6. "Late" binucleated erythroblast

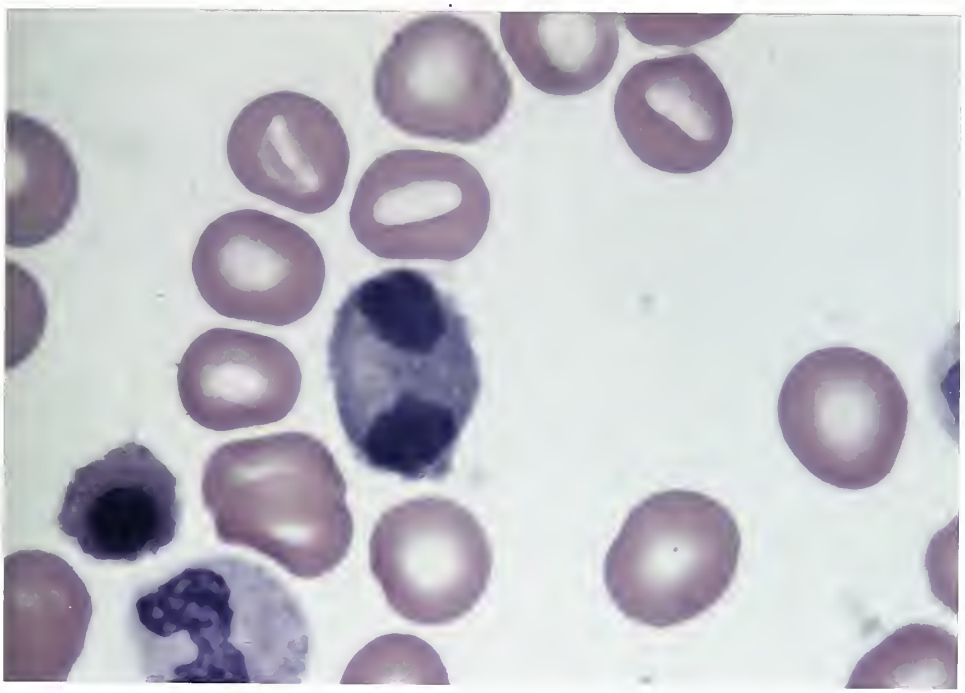

Figure 7. Late mitosis (erythroblast). Note elliptoid cytoplasm with eccentricity of the nuclei.

Figure 8. Late mitosis (erythroblast)

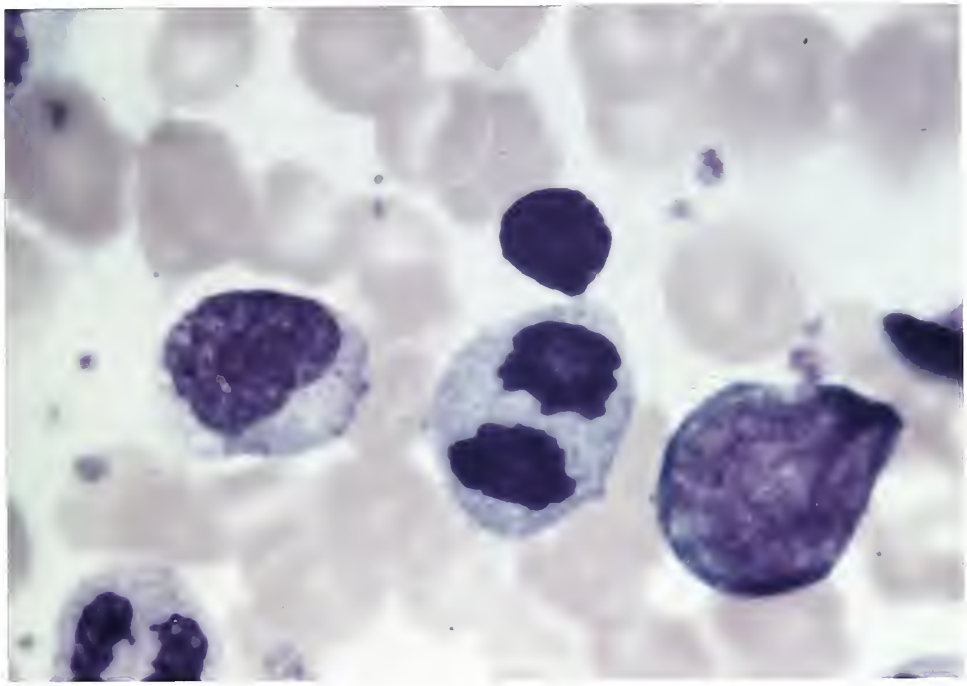

Figure 9. Late mitosis (erythroblast) Nuclear chromatin not uniformly condensed.

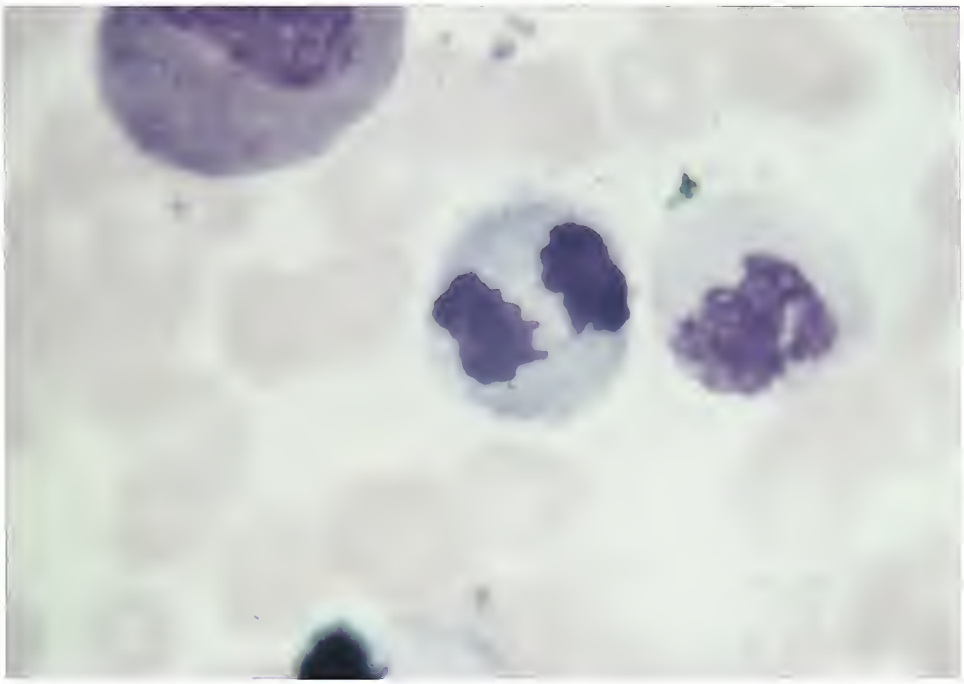

Figure 10. Late mitosis (erythroblast)

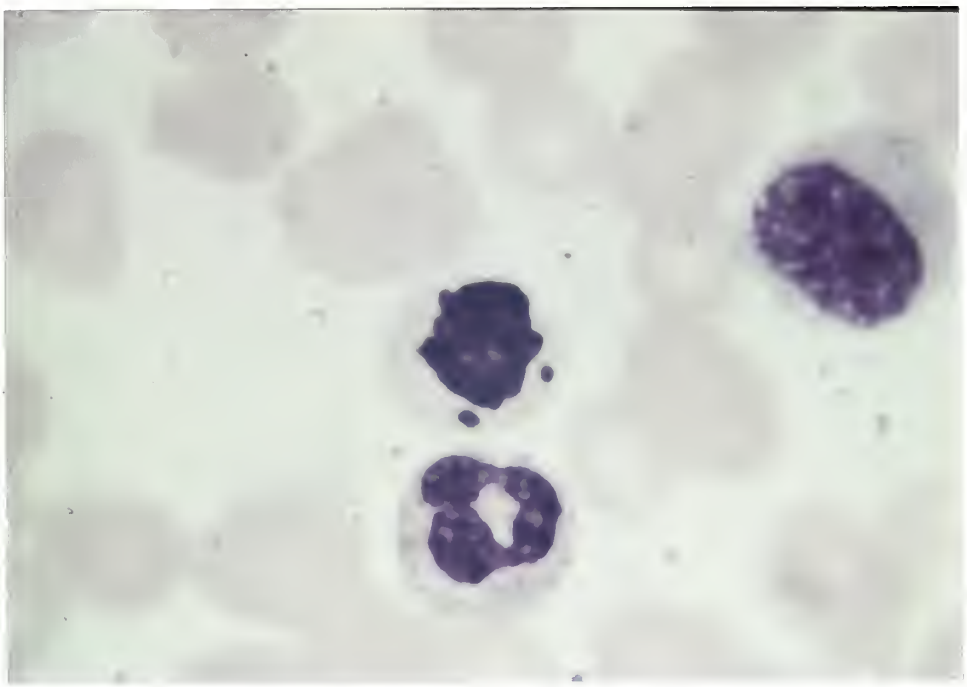

Figure 11. Karyomeres in an erythroblast

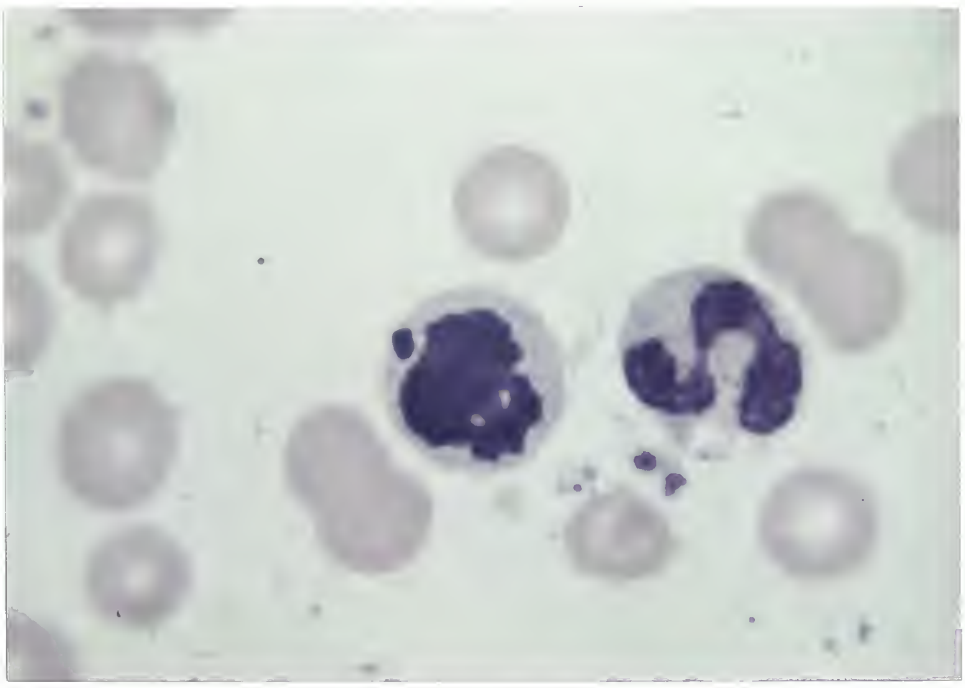

Figure 12. Karyomere

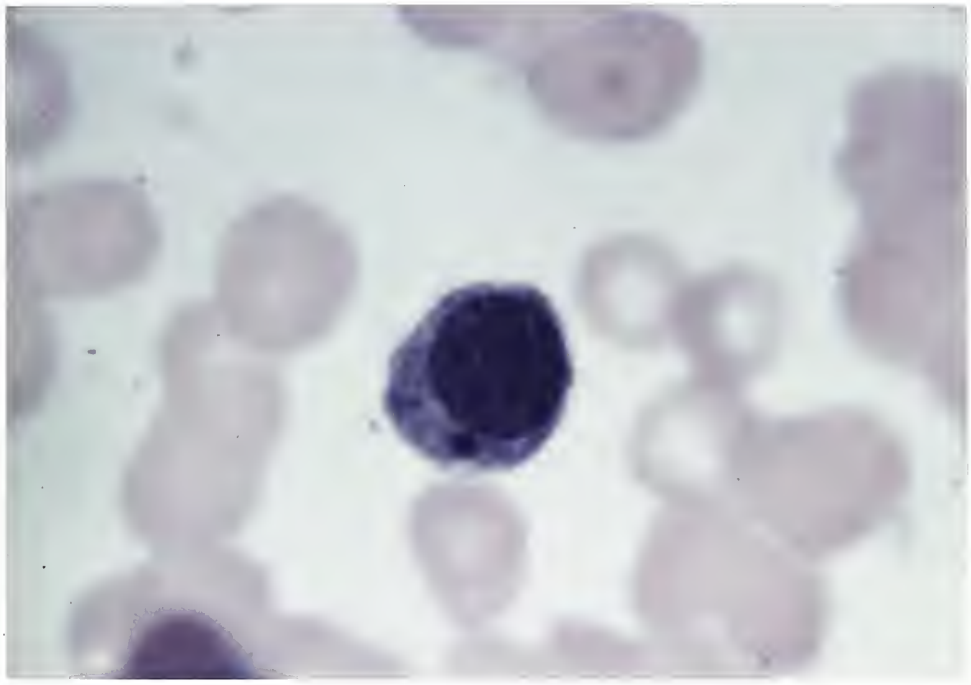

Figure 13. Karyomere

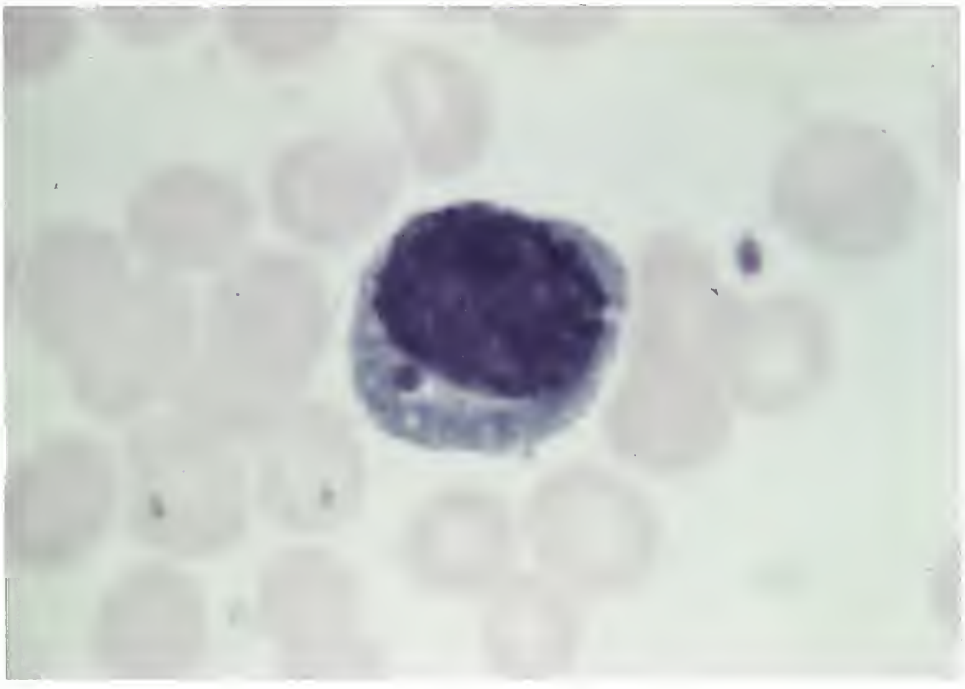

Figure 14. Karyomere

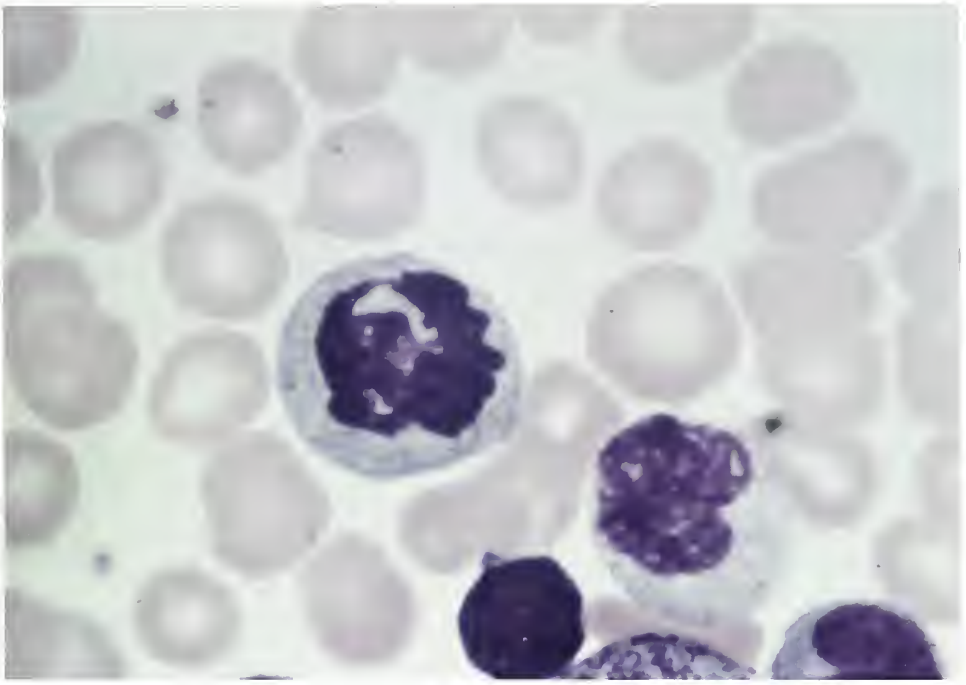

Figure 15. Mitotic "bridging"

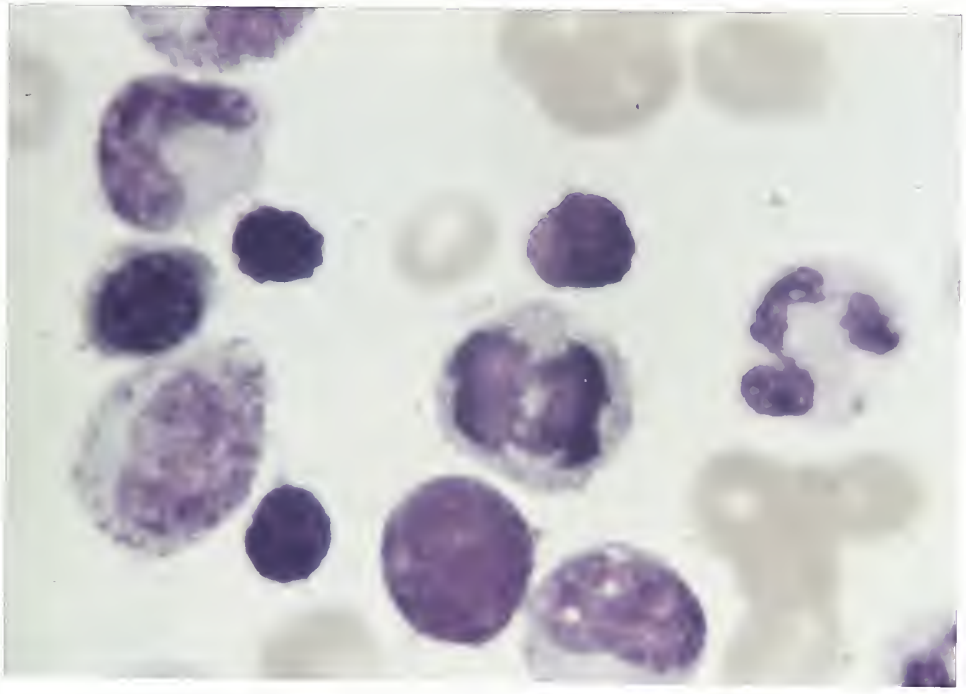

Figure 16. Mitotic "bridging"

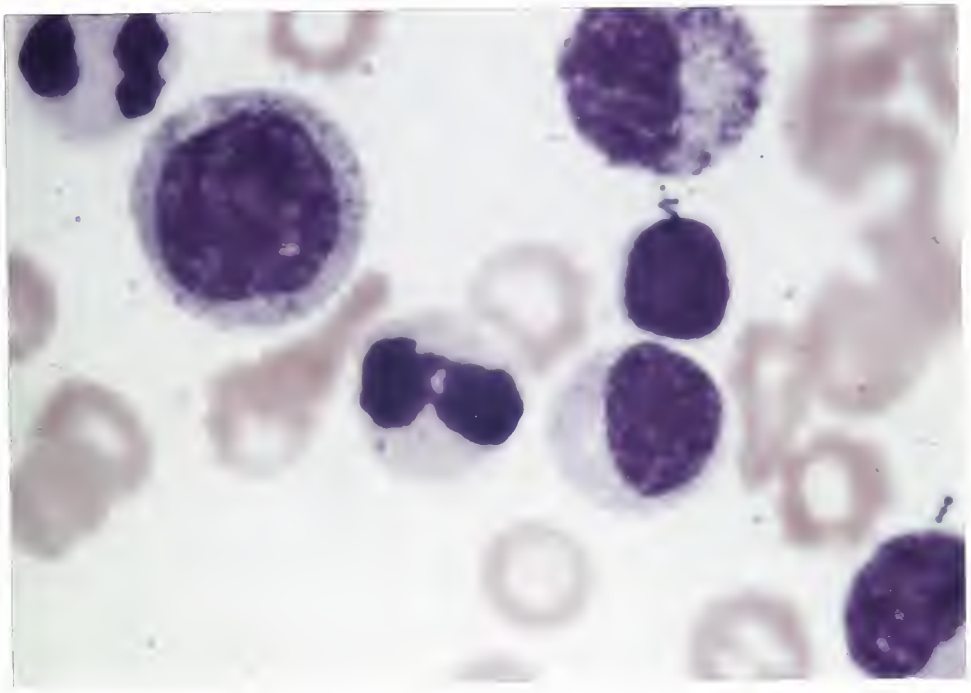

Figure 17. Mitotic "bridging"

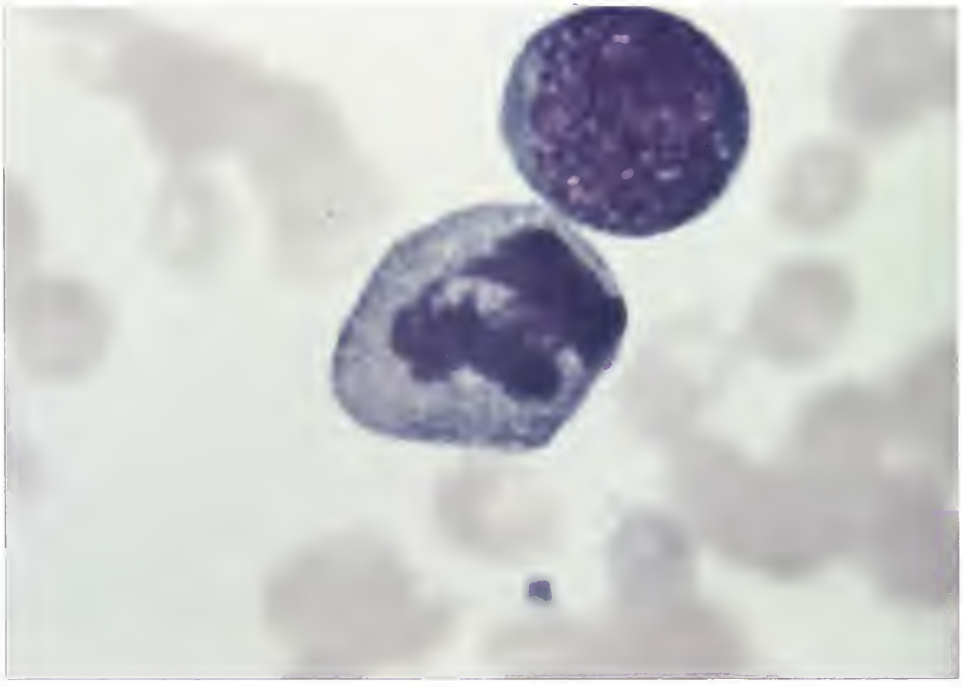

Figure 18. Mitotic "bridging"

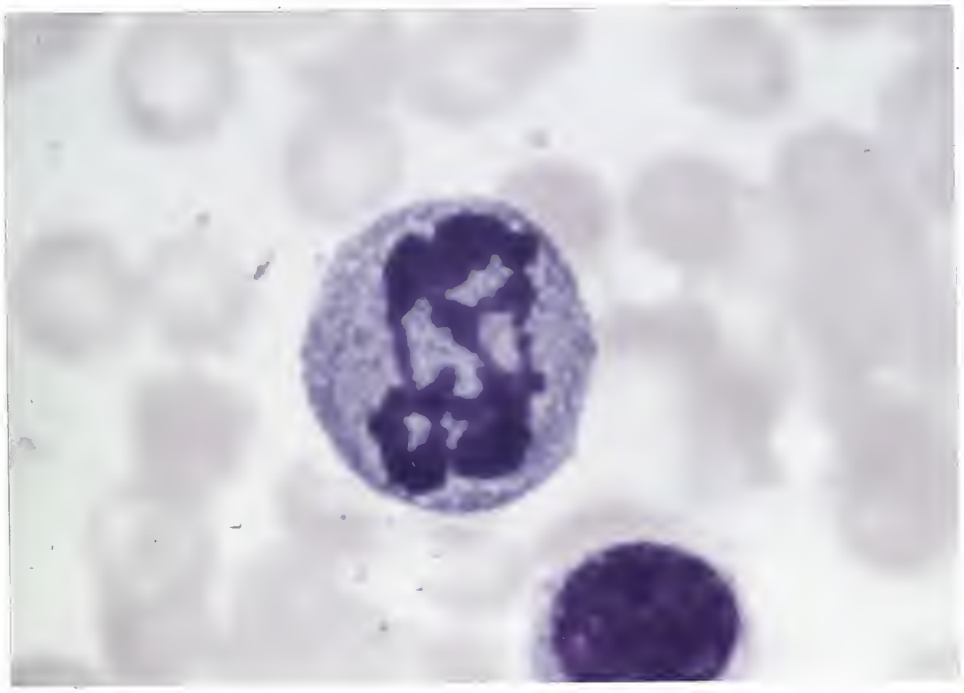

Figure 19. Bizarre mitotic "bridging"

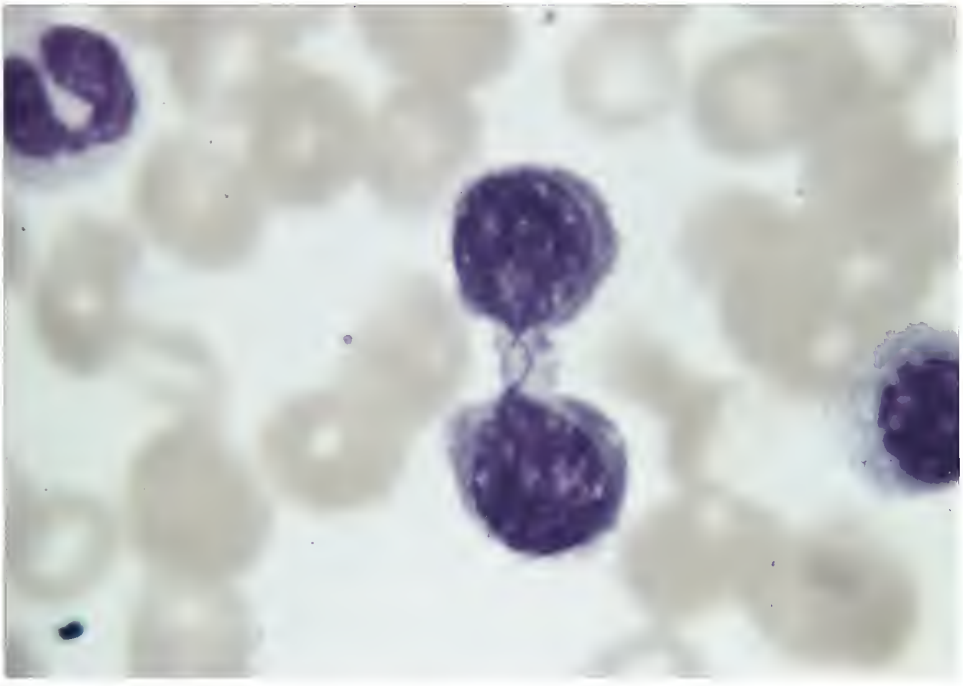

Figure 20. Internuclear Bridge.
Two erythroblasts with a bridge of chromatin sheathed in cytoplasm.

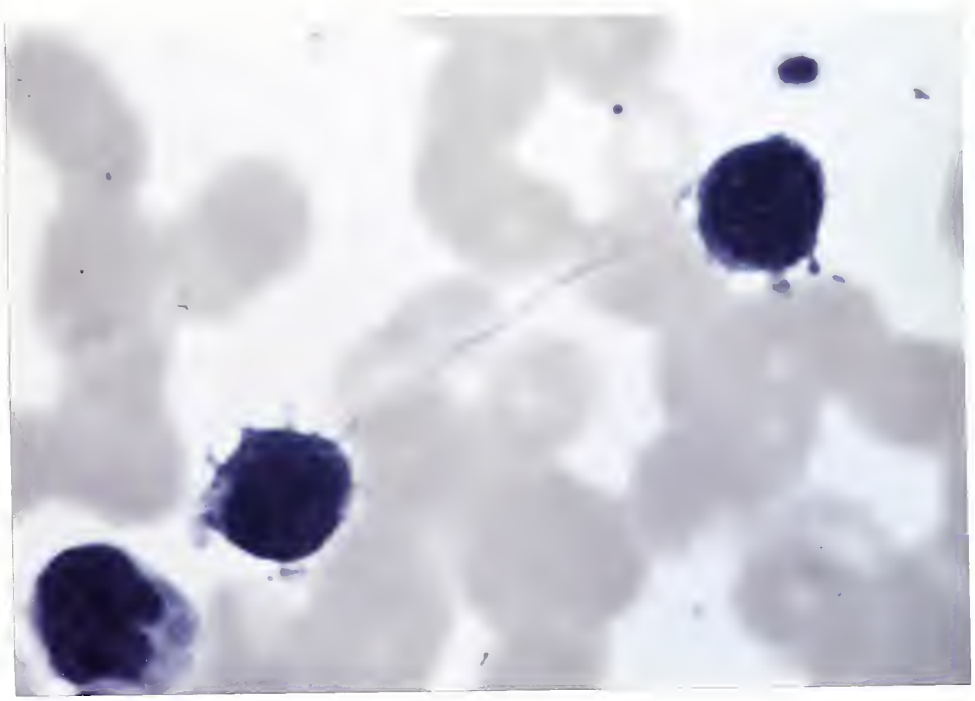

Figure 21. Internuclear bridge

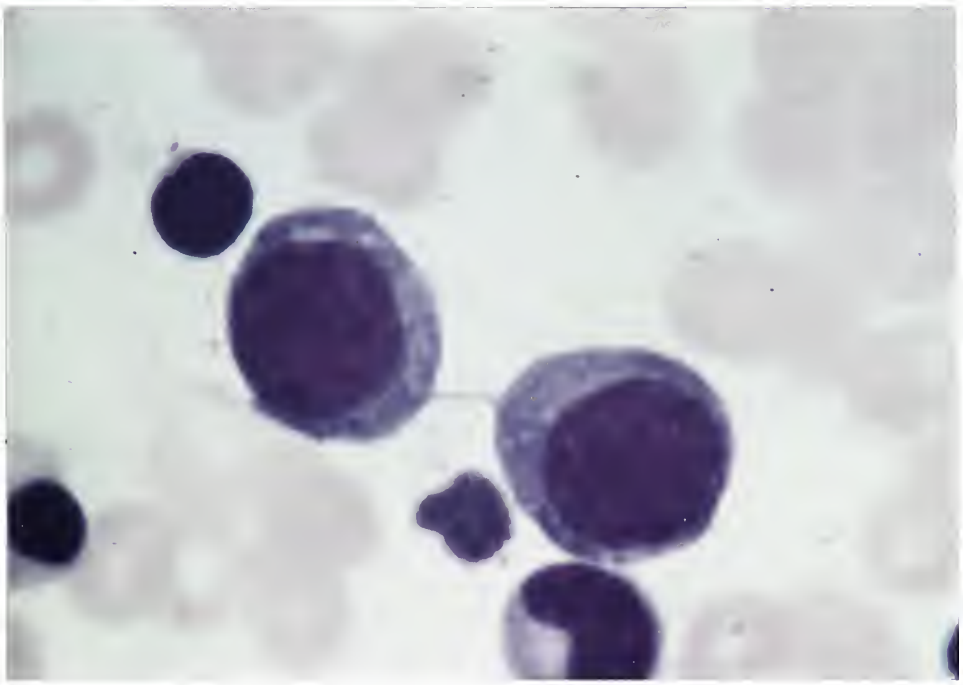

Figure 22. Internuclear bridge

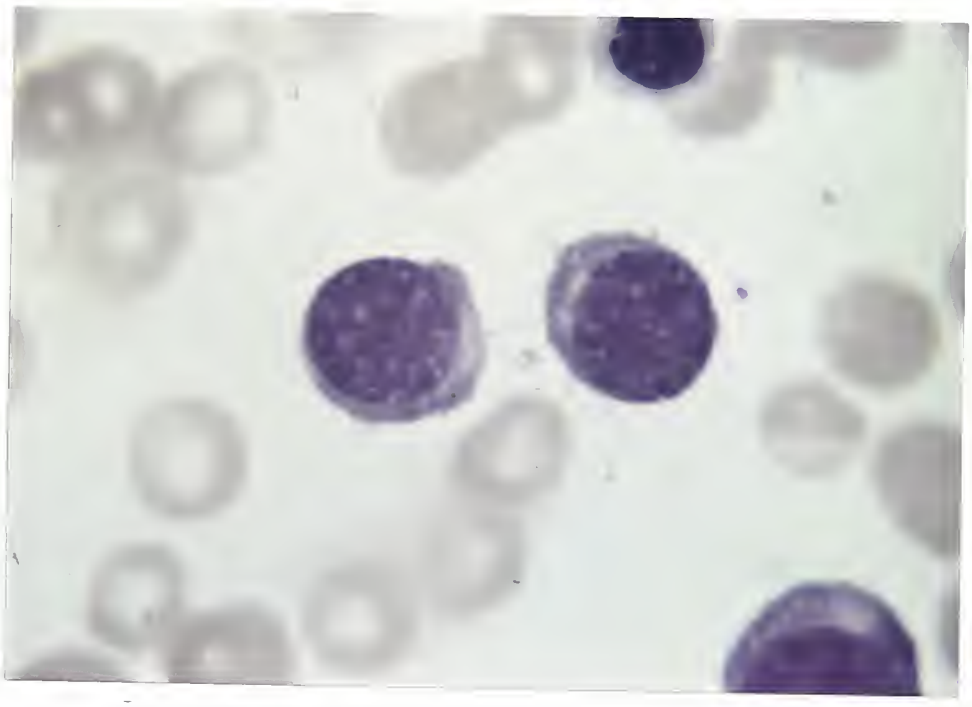

Figure 23. Internuclear bridge

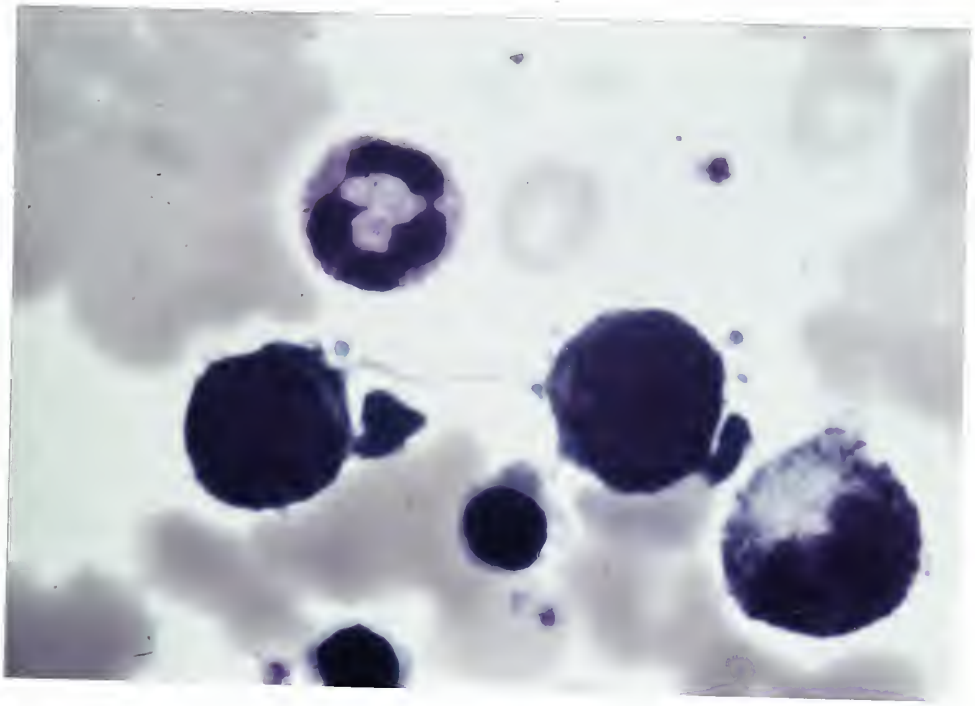

Figure 24. Internuclear bridge

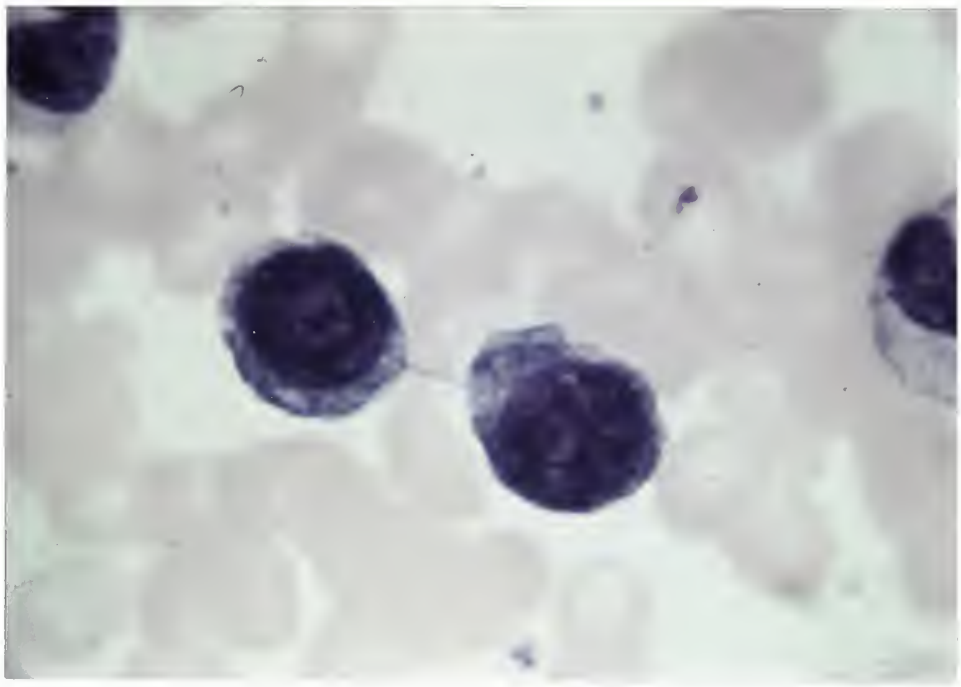

Figure 25. Internuclear bridge

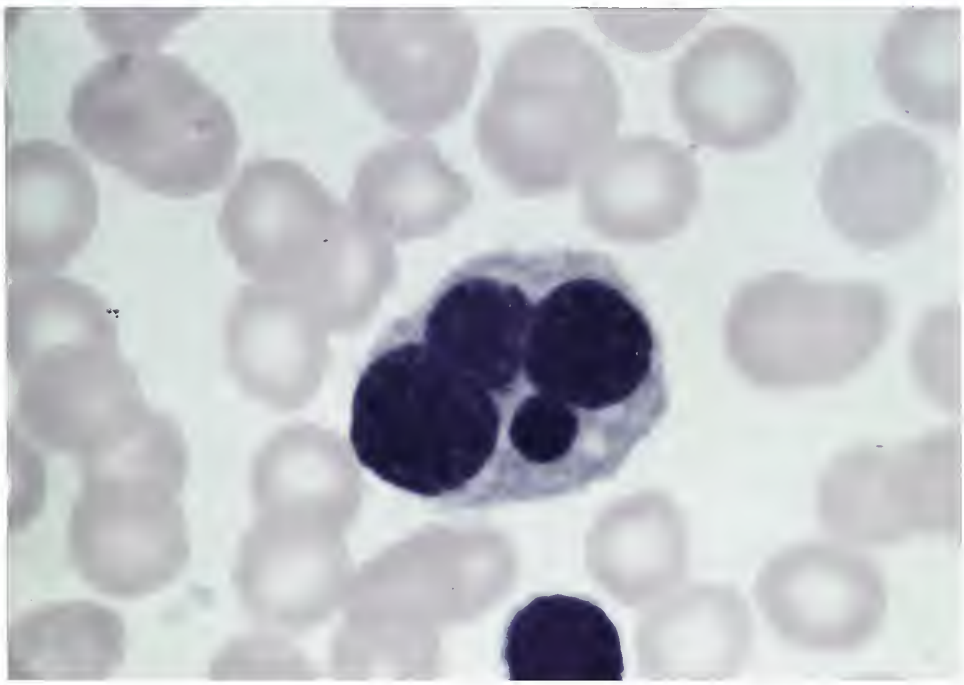

Figure 26. Multinucleated erythroblast

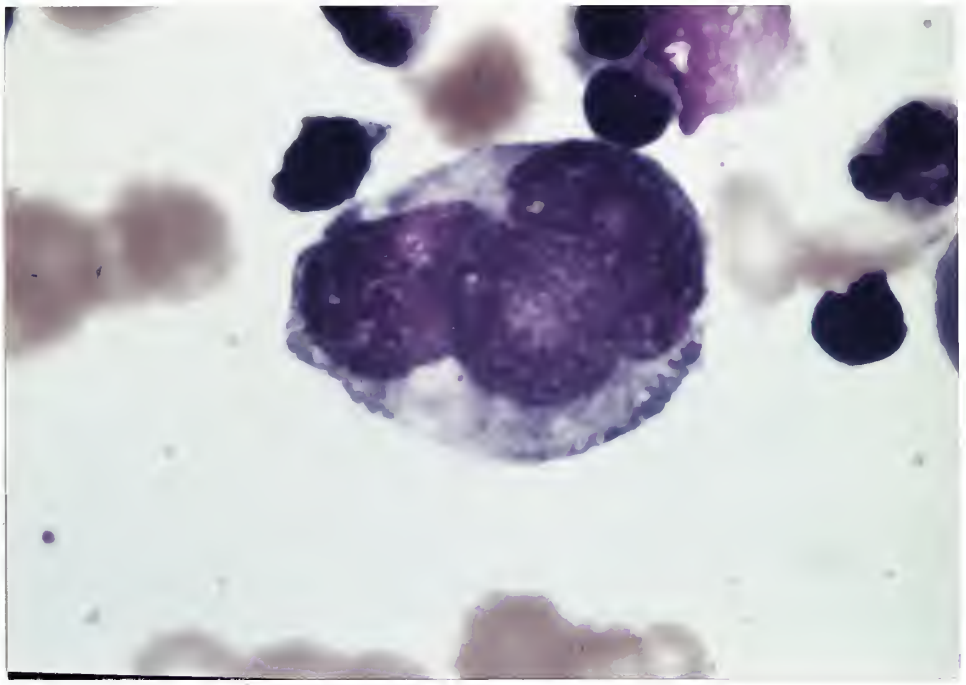

Figure 27. Multinucleated precursor (probably erythroid)

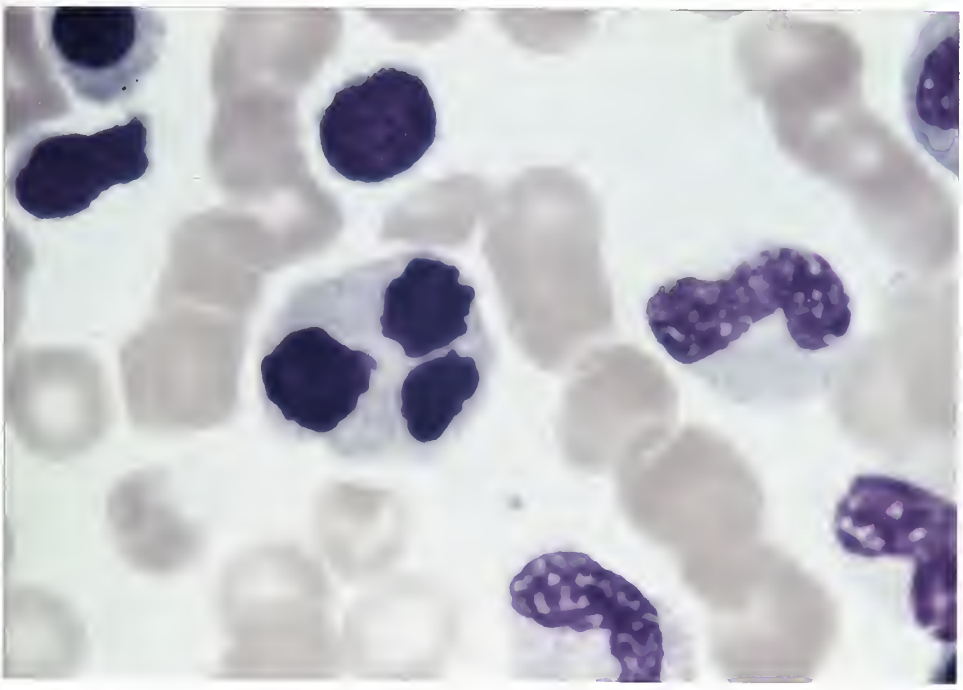

Figure 28. Trinucleated erythroblast

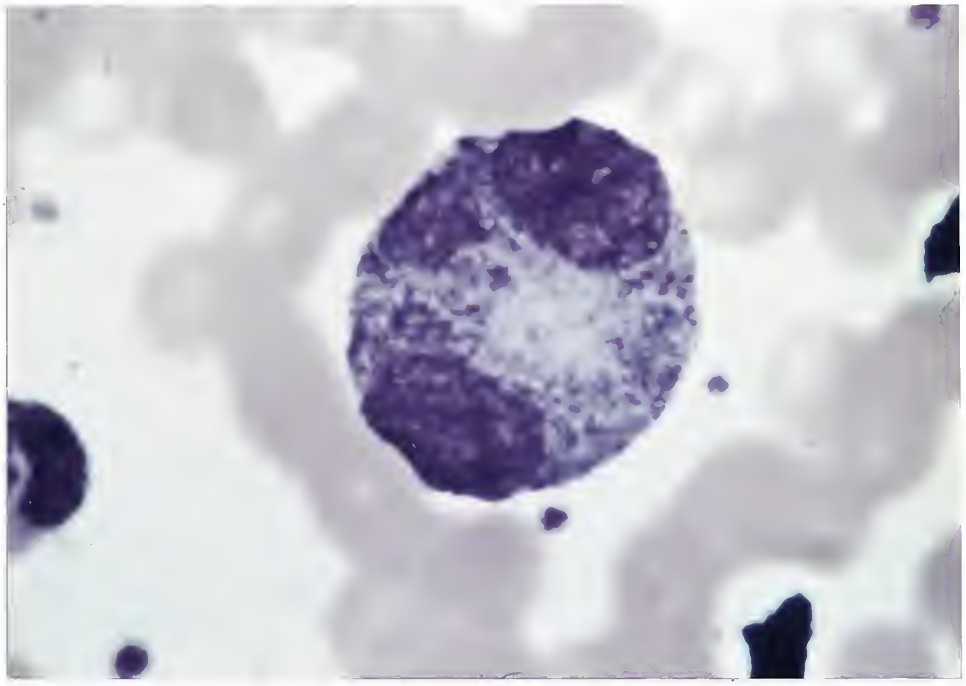

Figure 29. Trinucleated myelocyte

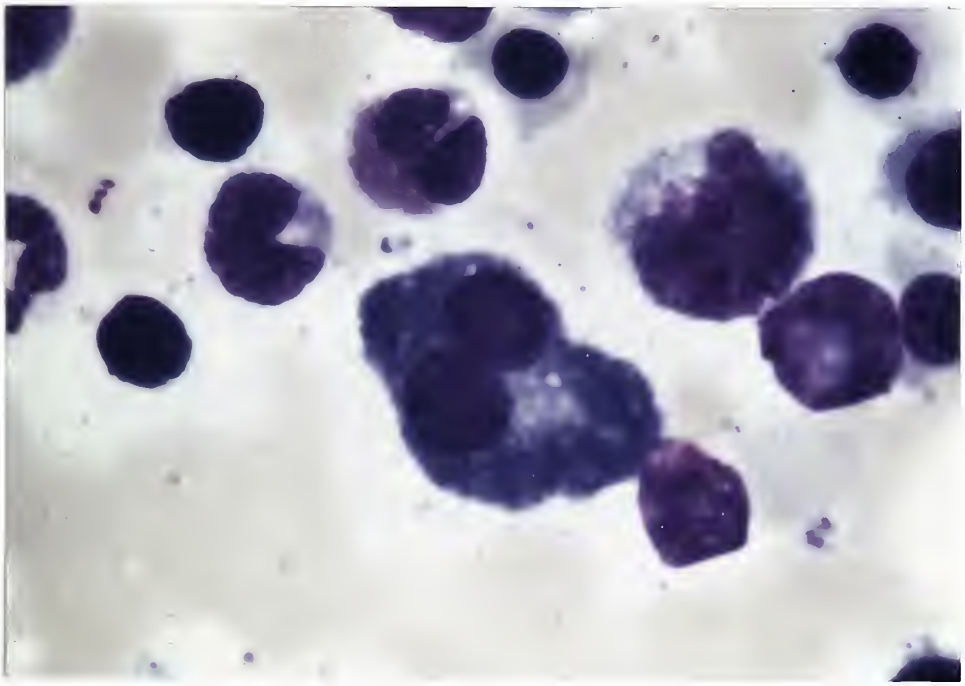

Figure 30. Binucleated plasma cell

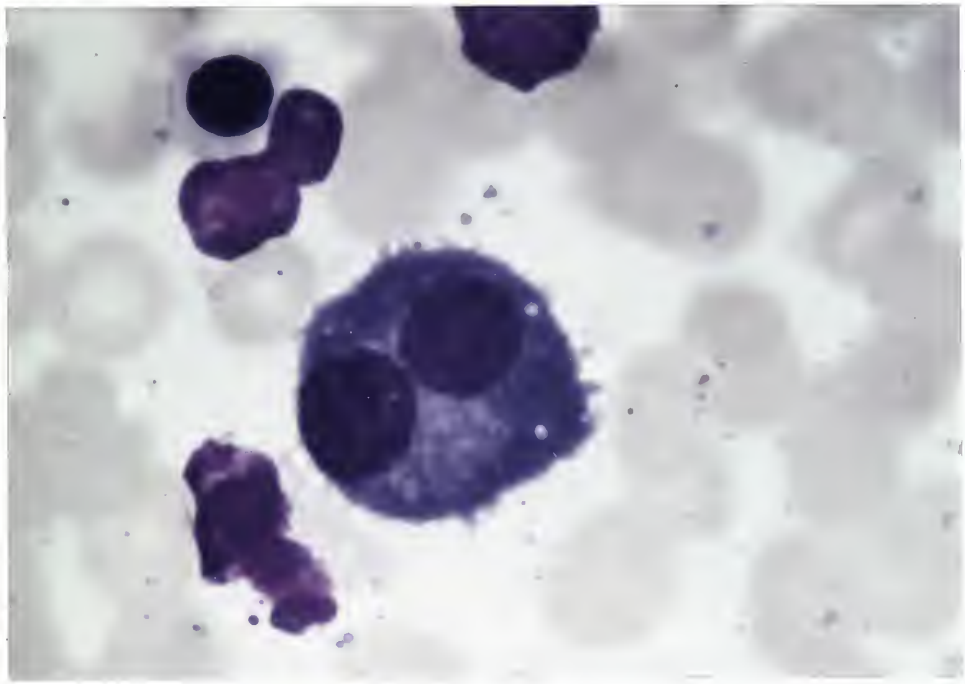

Figure 31. Binucleated plasma cell

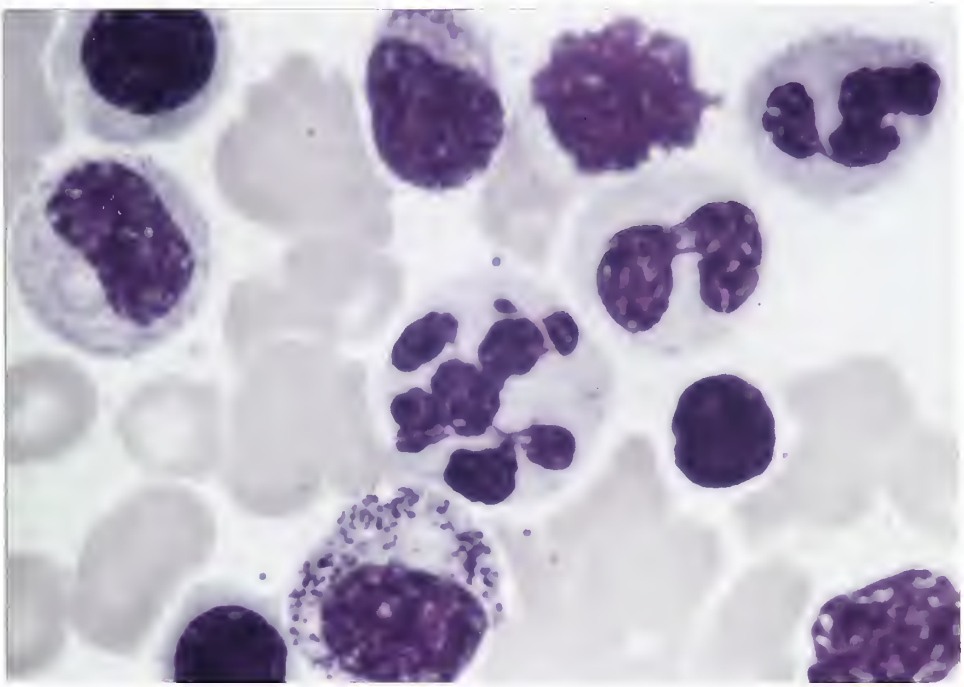

Figure 32. Hypersegmented neutrophil

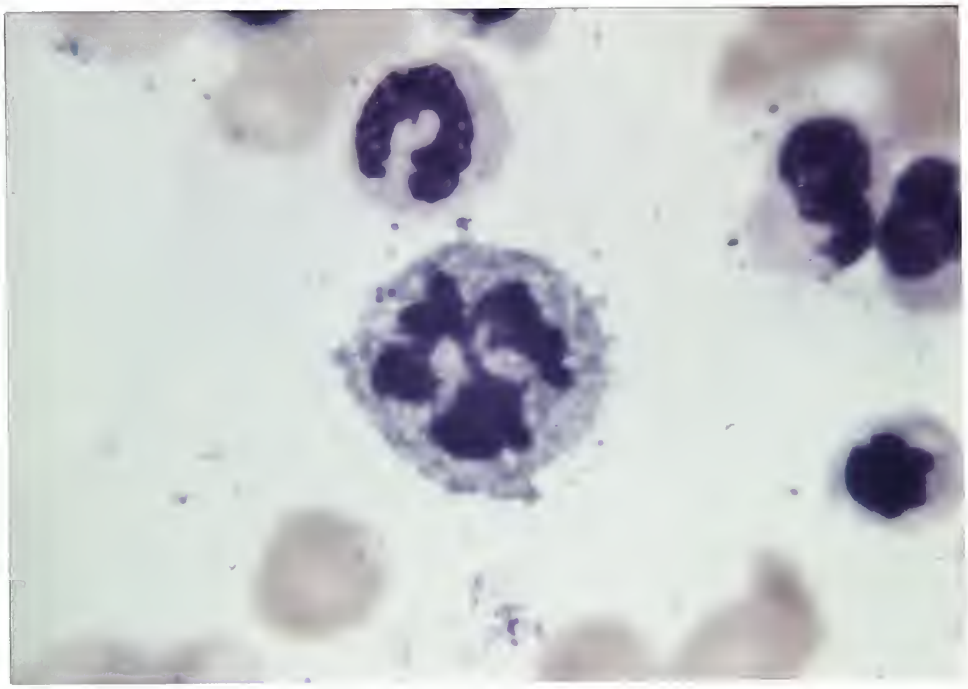

Figure 33. Multipolar mitosis

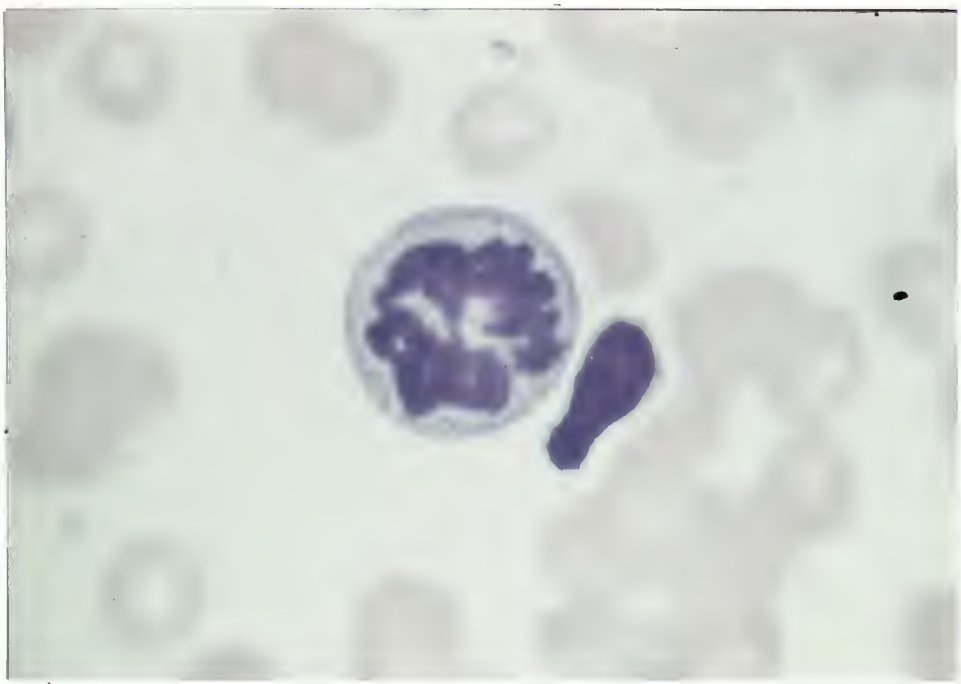

Figure 34. Multipolar mitosis

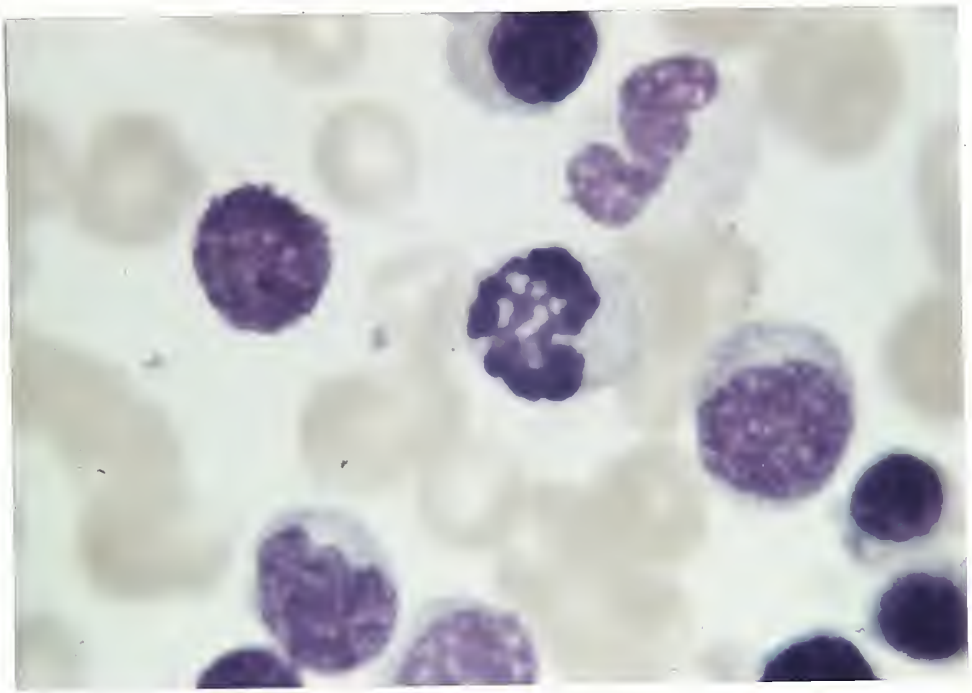

Figure 35. Multipolar mitosis

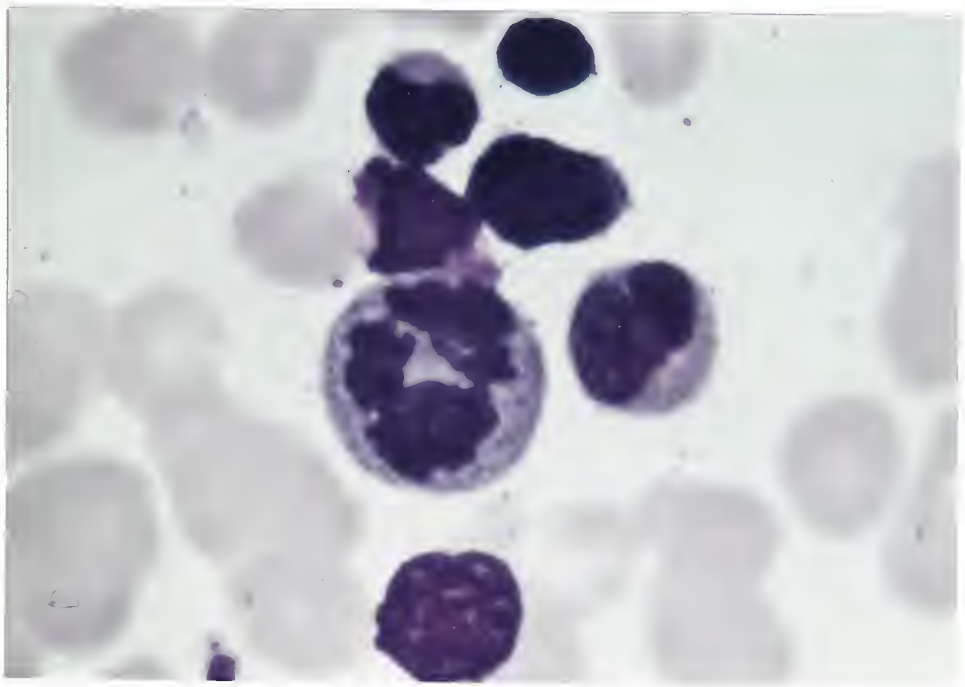

Figure 36. Multipolar mitosis

Section 5

DISCUSSION:

In evaluating the occurence of cytologic abnormalities in a bone marrow specimen prepared by ordinary coverslip technique, one should be aware of the variation in cellularity, structure, and staining properties from field to field within the same slide. In this study the majority of abnormalities were noted to be within the erythroblast series and were reported as a relative number, i.e. abnormal cells per 1000 erythroblasts. Thus variation in cellularity probably was not an important factor. The specific abnormalities most frequently noted were for the most part, independent of any staining properties and thus the present data is believed to be reliable.

The examination of bone marrow sections would have been most desirable and it would have been helpful if more "low dose" and control marrow smears had been available for study. Nonetheless, the availability of some controls of bone marrow smears from individuals exposed to a wide range of relatively high doses has made it possible to look for dose related effects. Observer bias was not a factor since all cytologic studies were done without knowledge of exposure.

Although cytologic abnormalities were abundant in the "late" bone marrows, no clear gradient with dose was established nor was there a statistical difference from controls.

The only convincing demonstration of the "late" effects of ionizing radiation, vis-a-vis abnormal bone marrow cytology, stems from a nuclear reactor accident at Oak Ridge in 1958 (18). Eight men were exposed to mixed gamma-neutron irradiation as the result of a critical excursion during the processing of waste Uranium^{235}. Five of the men received "high" dose exposure with estimated whole body irradiation between 236-365 rad. Three in the "low" dose group had estimated exposures of 68.5, 68.5, and 22.5 rad, respectively. Serial bone marrow aspirations were performed on this group during the immediate post-exposure period and were reported by Fliedner et al (30). Two types of cytologic abnormalities were described in these early marrows: (1) Cells injured directly and (2) "mitotically connected abnormalities" (noted in both mitotically active cells and interphase cells). For mitotic cells in ana- or teleophase, Fliedner described "bridges" of chromosomal material between separating fragments. He also described cells late in anaphase in which a chromosome or chromosomal fragment had strayed from the mitotic spindle, thus removed from further karyokinesis. Mitotically connected abnormalities in interphase cells included nuclear fragments (karyomeres), bi-nucleated cells and "giant" cells of the myelocytic series.

Three and one half years following the accident at Oak Ridge, followup bone marrow aspirations on these same individuals were obtained. Control specimens from four healthy men of comparable

age were also drawn. The results were reported in a second paper by Fliedner et al (29) . The most significant finding in these "late" marrows was an increased number of binucleated and, occasionally, trinucleated erythroblasts. An increased number of erythroblasts with karyomeres was also reported though its significance was not established. Amongst cells in mitosis they reported the frequent occurence of chromosomes or chromosomal fragments discarded from karyokinesis. Two examples of tripolar mitoses were noted in the course of counting 15,000 erythroblasts from the high dose group.

Fliedner et al. focused on the occurence of binucleated erythroblasts. They determined the frequency of binucleated red cell precursors per 1000 erythroblasts and reported a mean of 5.8 for the 5 men in the high dose group and 2.25 for the 2 men in the low dose group and 1.2 for the control cohort. While the difference between the high dose group and the control was statistically significant, that between the low dose and control was not. Our study did not demonstrate a statistical difference between exposed and controls. For our exposed group, the mean values for the frequency of binucleated erythroblasts per 1000 erythroblasts was considerably lower than the mean value for Fliedner's high dose group. It was thought that the difference between these data and Fliedner's might be due to different criteria for distinguishing late mitoses from binucleated cells. When reviewing these marrows, Cronkite (31) states that he considered

a cell to be binucleated and not in mitosis if he saw nothing connecting the two nuclei and if there was no significant constriction of the cytoplasm. Fliedner (32) relied on the nuclear chromatin structure, stating that a cell late in anaphase has not completed its nuclear membrane and the chromatin pattern is not yet distinct. Feeling that we had perhaps been too strict in our criteria for distinguishing binucleated cells, a statistical analysis was performed using the sum of total binucleated cells plus "late" mitotic cells. As can be seen in Table IV the addition of "late" mitotic cells to the unequivocally binucleated cells failed to impart statistical significance to our data.

Data from Fliedner et al. was derived from bone marrow aspirations obtained 3.5 years after exposure, while the data in this study was obtained from marrows 5 years post-exposure. It is conceivable that a five year hiatus allowed enough time for the marrows to normalize. Fliedner had reported mitotically connected abnormalities in 50% of cells from each of his acute, post exposure marrows. By three and one half years the incidence of mitotic abnormalities had dropped to approximately 4% of cells in each marrow. Though there is little basis for extrapolating this data, one might conjecture that, had bone marrow examinations been performed on Oak Ridge group at a point beyond three and one half years, differences between controls and exposed might have become insignificant.

Though Fliedner et al. emphasized the frequency of binuclearity in their exposed cohort, it should be mentioned that binuclearity is not a unique marker for radiation exposure. Erythroblastic binuclearity is one of the hallmarks of a relatively rare refractory anemia described by Wendt and Heimpel (33) in 1967. This unusual anemia was defined as Congenital Dyserythropoietic Anemia (CDA) and subsequently divided it into three types (34). Multinuclearity in the red cell series is a common feature in all three types, with a frequency of approximately 10-50% of the erythroblasts (35). Essentially, the characteristics of CDA are those of ineffective erythropoiesis; inappropriately low reticulocyte counts coupled with marked erythroid hyperplasia in the marrow and rapid clearance of radioactive iron from the plasma with subsequent poor utilization (36). The etiology of this dyserthropoiesis remains unknown.

Binuclearity (at a lower rate that in CDA) is also a common finding in bone marrows associated with other disease processes. Megaloblastic anemias are frequently associated with a striking erythroblastic multinuclearity (36-38). Schwarz (39) states that the frequency of binucleated cells in a given marrow roughly parallels the regenerative activity of the erythroid precursors and is a frequent finding in hemolytic anemias. This has been noted by others as well (38,40,41). Binuclearity is often reported in erythroleukemia (40,41). There have been spurious reports of binucleated erythroblasts in iron deficiency (41) as well as vitamin

E deficiency (42).

The significance of binucleated erythroblasts is obscure. Though associated with radiation injury as well as several disease states, they can also be a common finding in normal marrows. Berman (38) examined the marrows of 8 healthy individuals and noted the incidence of plurinuclear erythroblasts to vary from 1.0-5.1 per 1000 uninuclear erythroblasts. In the four controls of Fliedner et al. a mean incidence of binucleated erythroblasts was 1.2, while the mean for the high dose group was 5.1 per 1000. Both values are within the range of Berman's normals. Fliedner et al. noted this and states that a statistical comparison was only made with their own controls since they were studied in the same manner as their irradiated subjects.

Schwarz (39) observed that in normal marrows the most common binucleated erythroblasts were generally mature and close to denucleation, with the cytoplasm being orthochromatic or slightly polychromatic. He found it exceptional for a nuclei to retain proerythroblastic features. In the "late" bone marrows from Oak Ridge, Fliedner et al. emphasized the occurrence of "early" binucleated erythroblasts (corresponding to proerythroblasts and basophilic normoblasts) should be considered abnormal. The incidence of "early" binucleated erythroblasts in their high dose group was 1.4 compared to essentially zero in the low dose and control groups. Table gives the incidence of early binucleated erythroblasts

per 1000 in our study. The incidence is small for exposed subjects in all dose groups as well as the controls. There is an apparent gradient with dose but it is not statistically significant.

In our study the occurence of karyomeres was also a frequent finding in most of the marrows. Despite a dramatic increase in the 600+ rad group, a statistically significant gradient with dose was not established. As was mentioned, Fliedner et al. reported an increased number of erythroblasts with karyomeres, but absolute numbers were not reported. The term karyomere was used by Schwarz (39) to describe aberrant nuclear fragments. There is probably little difference between karyomeres and Howell-Jolly bodies other than extrusion of the nucleus in the latter. Discombe (43) demonstrated that Howell-Jolly bodies evolve from chromosomes that fall away from the mitotic spindle during abnormal mitosis. Presumably, at the completion of mitosis these fragments coalesce into smaller nuclear bodies. Bessis (44) has examined Howell-Jolly bodies with the electron microscope and from his studies it appears that they are surrounded by normal nuclear membrane.

In an animal study, Fliedner (21) irradiated rats and injected them with tritiated thymidine prior to sacrifice within the first 24 hours post exposure. He found that these aberrant nuclear fragments (karyomeres) were labeled, indicating that they still retained the capacity to synthesize DNA. He made the point that, in a functional sense, karyomeres must be considered to be

ectopic nuclear material and not mere remnants of karyorrhexis.

Though "bridging" between chromosomal material in mitotic cells is a well described sequela of acute radiation damage, the present study does not indicate that bridging can be used as a reliable marker of persistent radiation injury. Its incidence was quite rare and its absence in the control was insignificant. Fliedner et al. do not give exact figures but state that abnormal mitoses, including mitotic bridges and aberrant chromosomes, were as high as 50% in the acute marrow of the high dose group. In the "late" study, they report inter-chromosomal bridging only in the myelocytic cells.

Pluripolar mitoses were uncommon in our study and had no significance as a radiation marker. Fliedner et al. attached some importance to their finding of three independent tripolar mitoses in the high dose group, noting that Berman failed to observe a single pluripolar mitosis in 53,167 erythroblasts of 8 health controls. The fact that it is seen so infrequently and so inconsistently does not lend itself to a study of statistical significance, and leaves the observation of dubious importance.

The most interesting finding in our review of "late" marrows was the occurence of internuclear bridges in seven of the survivors. Though found mainly in the heavily irradiated subjects, its incidence was once again too infrequent to be used as a reliable radiation marker and even less so as a dosimeter.

Internuclear bridging is a rare finding. It has been reported in the Congenital Dyserythropoietic Anemias. Internuclear bridges serve as one of the distinguishing features of the so-called-type I CDA (35,36). Lewis et al. (41) have done EM studies on CDA, type I, and have demonstrated a narrow sheath of cytoplasm surrounding the chromatin like strand of material that bridges the nuclei of the two, otherwise distinct, erythroblasts. This phenomenon is dramatically demonstrated in figure 20 from our study.

It is possible that these internuclear bridges found in interphase cells are the end result of a process which began as chromosomal bridging in a mitotic cell. In autoradiographs of marrow obtained from subjects exposed to large doses of ionizing radiation and then incubated in vitro with tritiated thymidine, Fliedner (45) reported one instance in which an internuclear bridge was labeled along with the two nuclei which it connected. The inference drawn was that the internuclear bridge was still functional chromatin-like material, able to synthesize DNA in conjunction with both nuclei.

As was mentioned, criteria for identification of internuclear bridges were very strict. Pure cytoplasmic bridges were not included. Fliedner does not appear to differentiate between internuclear bridging and cytoplasmic bridging between two interphase erythroblasts. In his study of late marrows he states that he did not quantitate the apparent increase in red cell precursors with cytoplasmic bridges between cells in the high

dose group. Lewis (41) has investigated cytoplasmic bridging with the aid of an electron microscope and has demonstrated what appears to be a spindle bridge consisting of microtubules, sheathed by cytoplasm, running between two erythroblasts. Schwarz also noted intercellular cytoplasmic bridging and attributed it to "residual interzonal fibers".

In our study, intercellular bridging, purely by cytoplasmic extension, was not enumerated because it was felt that this could be an artifact of preparation. Without the aid of an electron microscope to clearly demonstrate micro-tubular structures streaming down a tube of cytoplasm from one tube to the next, one could not be sure that he was not simply viewing 'sticky' membranes from two cells that had been juxtaposed during preparation. On the other hand, the observation of chromatin material connecting two separate interphase erythroblasts is unlikely to be artifactual. Though internuclear bridging was not observed frequently enough in the exposed cases to be a statistically valid dosimeter of radiation injury, it remains a curious finding in these 'late' marrows.

The "Mitotic Index" is an indication of the proliferative capacity of the marrow (46) . In an earlier paper, Fliedner points out that determining the mitotic indices from ordinarily prepared bone marrow smears is inaccurate. Mitosis should be properly enumerated in Feulgen squash preparations. In our study, with Fliedner's caution in mind, mitoses were recorded in the course of counting 4000 erythroblasts. Without regard to specific cell type, our rough

mitotic index has a statistically suggestive gradient with increasing dose (Table VI). The rough mitotic index was 6/1000 for the control cohort and 10/1000 for the 600+ rad group. Both animal and plant experiments have demonstrated that exposure to ionizing radiation can cause a lengthening of the mitotic interval. If a cell, damaged by radiation, were to spend a longer time in mitosis, the probability of observing an anaphase or teleophase cell at any one point in time would be increased. Thus, as Fliedner points out, radiation injury might give a misleadingly high mitotic index.

Because the mitotic index in our study was determined without Feulgen-squash preps and the different cell lines in mitosis were not differentiated, little significance in terms of radiation injury can be appropriately attributed to the suggestive gradient with dose. In his study of the Oak Ridge survivors, Fliedner did prepare Feulgen-squash preparations on the late marrows and the mitotic indices were not found to be statistically different from the controls.

The only other significant report on persisting bone marrow cytologic abnormalities stem from an accidental exposure to fallout radiation in the Marshall Islands in 1954. The incident occured during a nuclear test in the Marshall Islands (Bikini lagoon). Full details of the incident and the subsequent medical findings from the people exposed are contained in a twenty year review (17) . The most heavily exposed population was a group of

64 people inhabiting Rongelap Island. Their entire exposure was in the form of fallout with radiation with a maximum estimated whole body dose of 175 rad. Bone marrow aspirations from four exposed and two control subjects were obtained nine years after the accident. Conard notes that, in addition to erythroid hyperplasia, there were "abnormalities of the chromatin material" with binucleation and an increased frequency of mitotic figures in the normoblastic series." Neither absolute numbers nor statistical significance was reported..

During the course of this accidental fallout in the Marshall Islands, 23 Japanese fisherman aboard the fishing vessel "Fukuryu-maru" (the "Lucky Dragon") were also subjected to fallout from the atomic detonation approximately 90 miles away. Kumatori (47) has reported on the bone marrow findings in this group of men, exposed to whole body radiation in the range of 170-700 rad. Of note, "late" marrows were aspirated on 14 exposed and 4 controls in 1964, 10 years after the initial exposure. Kumatori reviewed the marrows in a manner similar to that of our study. They also failed to demonstrate a significant difference between exposed and control marrows for binucleated erythroblasts (mean of 2.0 for exposed versus 1.6 for controls). Three individuals in the exposed group had a relative increase in karyomeres in comparison to both controls and others in the exposed group. No other cytologic abnormalities were reported.

Section 6

CONCLUSION:

From this study it appears that "late" bone marrow cytology is not a reliable dosimeter or marker of radiation injury. A cohort of survivors was used in which many had been exposed to very high doses of ionizing radiation. A significant gradient was not established for any of the abnormalities in bone marrow cytology that have been associated with both acute and persistent radiation injury. One interesting finding was the occurence of internuclear bridges in seven of the marrows from exposed subjects. It is conceivable that this cytologic aberration could be used as a marker of radiation injury but the occurence is too infrequent to be of value as a biologic dosimeter.

The findings of this study also suggest that a gradual disappearance of radiation induced late bone marrow changes continues for periods of three to five years or more following acute radiation exposure in the high dose range.

PART II

THE LONG TERM EFFECTS OF RADIATION ON ABSOLUTE LYMPHOCYTE COUNTS IN THE ADULT HEALTH STUDY SAMPLE- HIROSHIMA - NAGASAKI, JAPAN

Section I. INTRODUCTION

A study was undertaken to tabulate absolute lymphocyte counts in a large population of atomic bomb survivors in addition to non-exposed controls. The data spans a sixteen year period beginning twelve years after exposure. An attempt was made to isolate possible perturbations in absolute lymphocyte counts due to persisting radiation injury and/or age.

Section 2

MATERIALS:

The Adult Health Study Sample

The Adult Health Study Sample (AHS) is the major population of people who are evaluated by both clinical and laboratory parameters at RERF. The AHS includes heavily irradiated survivors and controls matched to age, sex and city. Subjects for the AHS were chosen from a 1950 A-bomb survivors survey, in addition to other censuses, and were divided into four groups:

GROUP I: survivors located less than 2000 meters from the hypocenter who reported acute radiation symptoms (n = 4993)

GROUP II: Survivors located less than 2000 meters from the hypocenter who did not report acute radiation symptoms. (n = 4987)

GROUP III: survivors located 3000-3499 meters from the hypocenter in Hiroshima and 3000-3999 meters in Nagasaki. (n = 4990)

GROUP IV: Not in the city (NIC) at the time of blast (ATB). (n = 4992)

The subjects in Group I, by nature of their high exposure comprise the center of interest in the AHS. The other three groups were conceived in relation to it, matching closely with regard to age, sex, and number. In 1958, biennial examinations were initiated for this cohort of approximately 20,000 people. These examinations were conducted at ABCC facilities in both Hiroshima and Nagasaki. In addition to extensive history taking and physical examination, routine laboratory analyses (complete blood counts, urinalysis, etc.) were performed on all subjects. Where indicated, further testing was undertaken. Participation in this study was on a voluntary basis. Belsky, in his review of the first five cycles of examination (48) notes "....concern over health, cultural inclination toward group participation, fear of unknown radiation effects, reward of a hopefully normal examination, etc, all play a part (in continuing participation)...". The eighth examination cycle (1974-76) is currently being completed. All data from the clinical laboratories, spanning the first seven examination cycles, is on magnetic tape and accessible through an IBM 1440 computer, on site in Hiroshima.

Section 3

METHODS:

Data concerning lymphocyte counts was stored in the form of total white blood cell counts, coupled with corresponding white cell differentials. This data was easily converted to absolute lymphocyte counts by the simple calculation:

ABSOLUTE LYMPHOCYTE COUNT = total WBC X % lymphocytes

Data from examination cycle I (1958-60), IV (1964-66), and VII (1970-72) were analyzed independently. The data was tabulated in a matrix fashion with age (00-29, 30-39, 40-49,...) on the abscissa and T65D dose (NIC, 0-9, 10-49, ...) on the ordinate. As indicated below, each entry into the matrix consisted of: n - (the number in that coordinate), mean - (mean absolute lymphocyte count for that number), and S.D, - (the standard deviation).

```
      :.....................00-29.............AGE
      :
      :
      :
      .
      .
      .
      .                      n = 197
DOSE  100-199r             mean = 2086
      .                      SD = 758
      .
      .
      .
      .
```

A pure "aging" effect was looked for in the non exposed group (NIC). Both a radiation and an "aging" effect was sought for in the exposed groups.

Section 4.

RESULTS:

A. The effect of previous radiation exposure on the absolute lymphocyte count.

The results of the three examination cycles analyzed are summarized in Tables I-III. There appears to be no relation between the magnitude of the absolute lymphocyte counts and the extent of radiation exposure. Figures (cycles I, IV, and VII respectively) represent this data in graphic fashion. One can control for age with these graphs by selecting a specific bar pattern and following it along the abscissa. For all three cycles, with age controlled, there is no consistent relationship between radiation dose and the absolute number of lymphocytes.

B. The effect of AGE on the absolute lymphocyte count.

Figures 4-6 are taken from Tables I-III respectively. They graphically demonstrate a significant diminution in the absolute lymphocyte counts in the 70+ age group. This finding holds true for all three examination cycles and for all groups, whether heavily irradiated or unexposed (i.e. not in city ATB). Table IV analyzes the data in a different fashion, with the mean absolute, lymphocyte counts transformed by $\sqrt{\ }$ (49) . For each of the three cycles, the mean lymphocyte count for the 70+ age group is significantly less ($\rho < .0001$) than that of all individuals aged 0-69, combined.

FIGURE I

EFFECT OF RADIATION ON ABSOLUTE LYMPHOCYTE COUNT

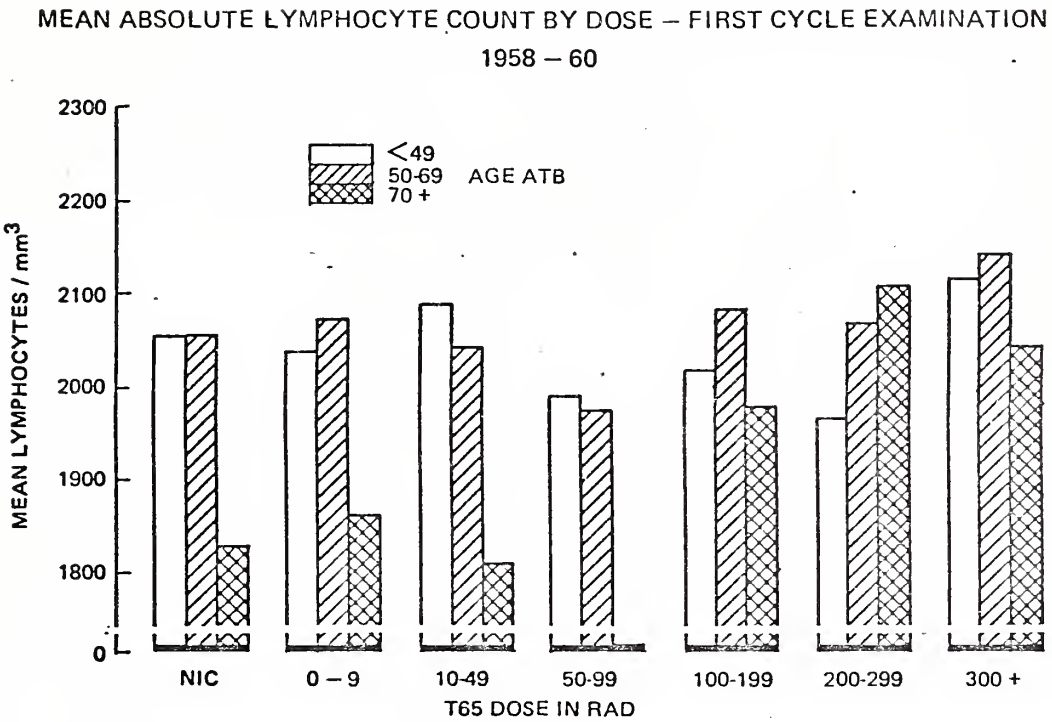

FIGURE 2

EFFECT OF RADIATION ON ABSOLUTE LYMPHOCYTE COUNTS

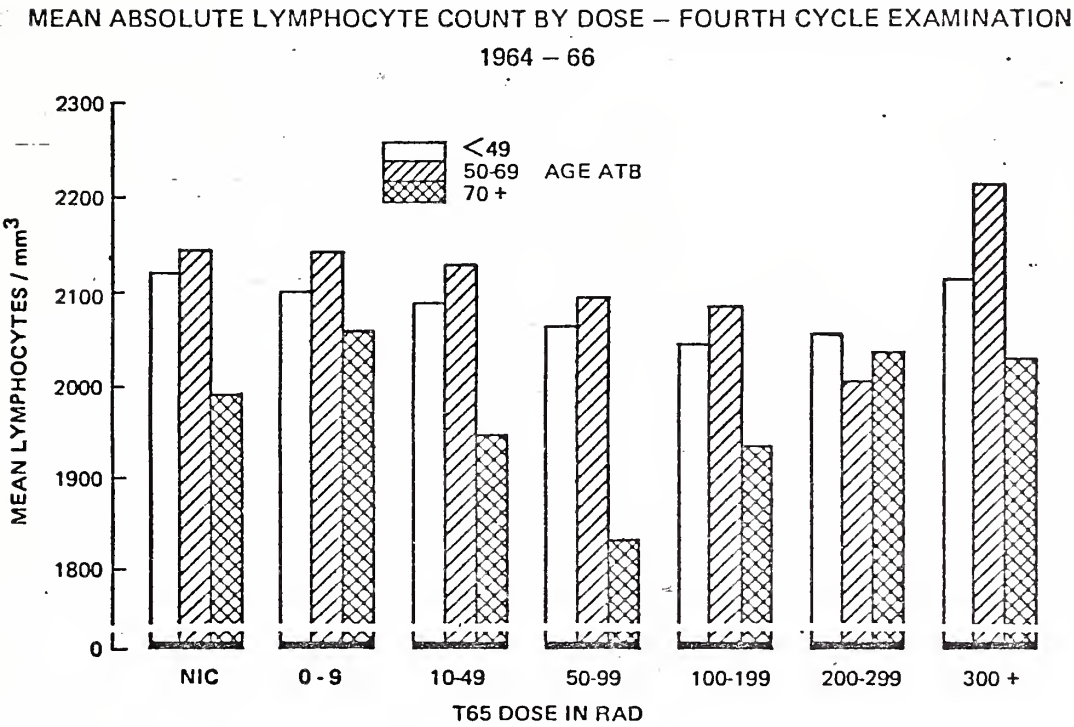

FIGURE 3

EFFECT OF RADIATION ON ABSOLUTE LYMPHOCYTE COUNTS

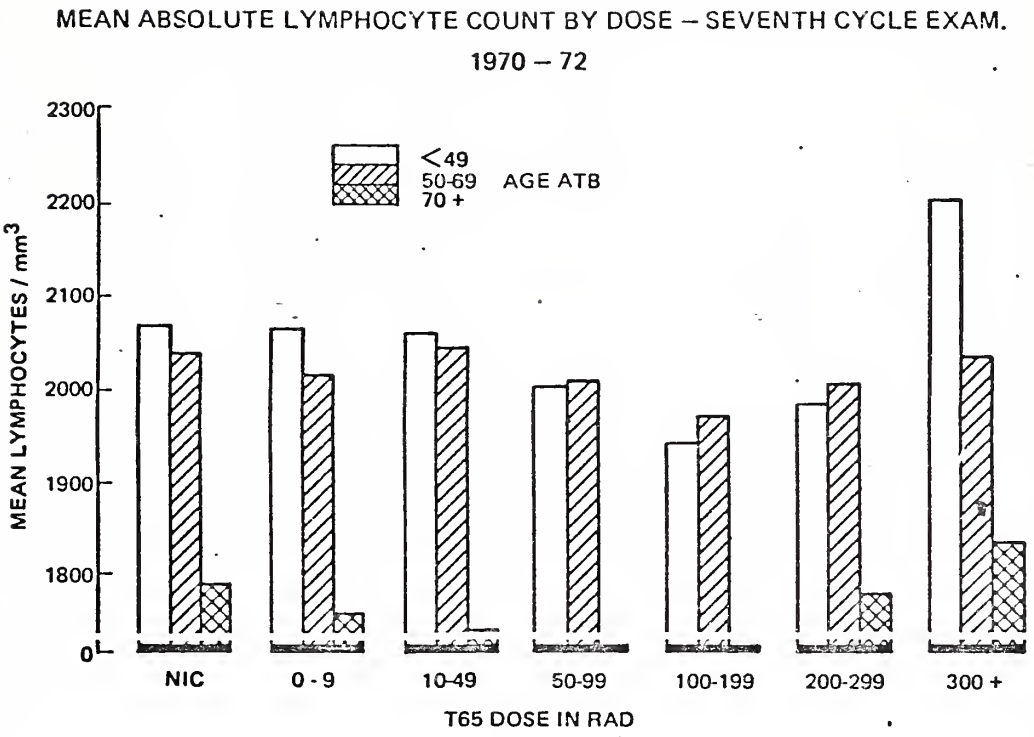

FIGURE 4

EFFECT OF AGE ON ABSOLUTE LYMPHOCYTE COUNTS

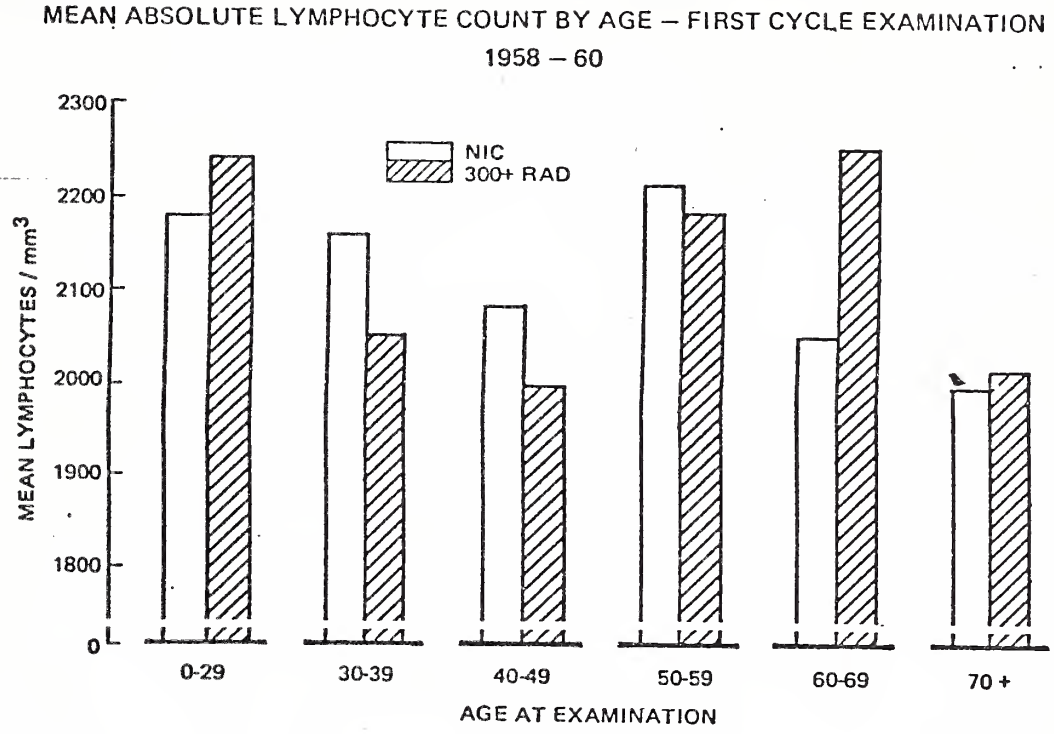

FIGURE 5 EFFECT OF AGE ON ABSOLUTE LYMPHOCYTE COUNTS

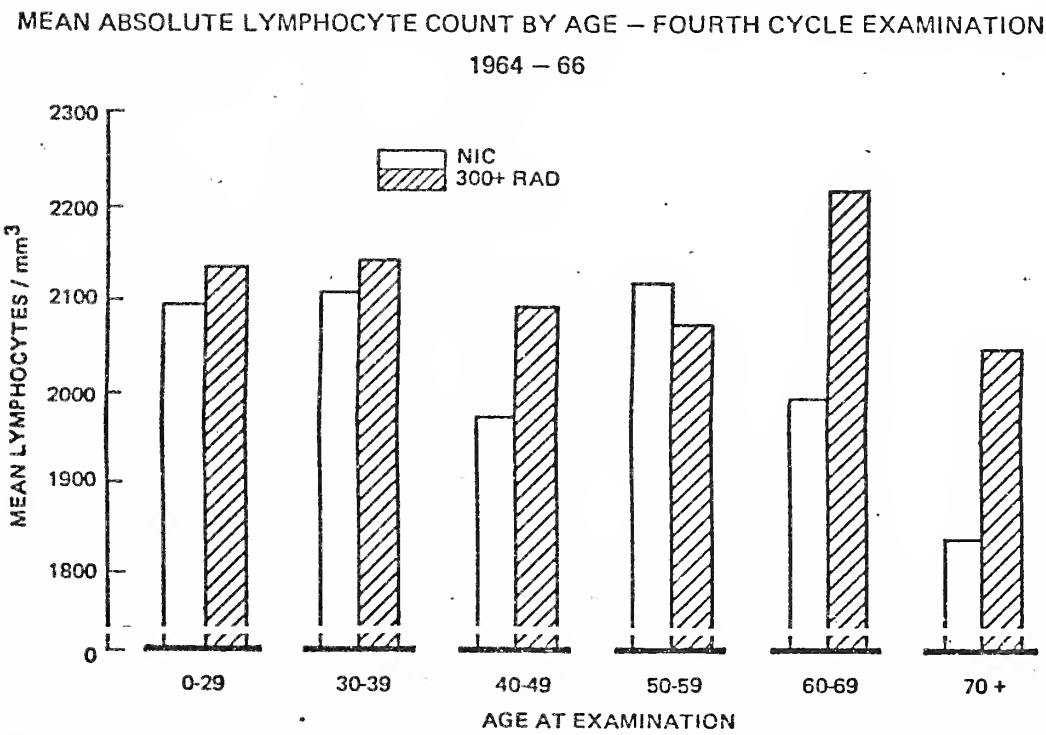

FIGURE 6

EFFECT OF AGE ON ABSOLUTE LYMPHOCYTE COUNTS

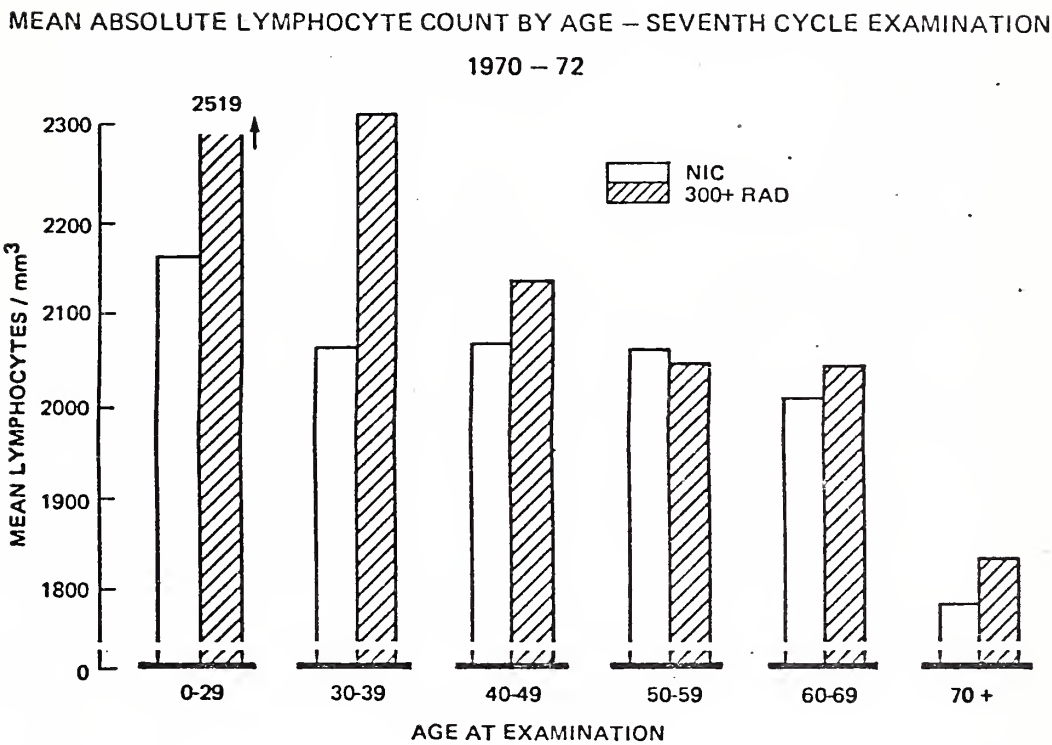

TABLE I

ABSOLUTE LYMPHOCYTE COUNTS, FIRST CYLCLE EXAM (1958-60)

T65D EXPOSURE DOSE (rad)	AGE 0-29	30-39	40-49	50-59	60-69	70+	TOTAL
NIC	607 2180 744	740 2111 750	559 2078 739	580 2216 810	393 2055 834	130 1996 681	3009 2127 769
0-9	879 2138 757	875 2041 713	650 2131 748	739 2172 840	517 2103 855	181 2060 806	3841 2113 780
10-49	364 2105 778	372 2105 744	386 2063 742	454 2165 805	287 2088 815	125 1949 851	1988 2098 782
50-99	169 2096 727	231 2068 728	162 2029 751	214 2167 866	138 1980 698	44 1838 629	958 2065 759
100-199	197 2086 758	283 2059 740	158 1966 743	204 2122 787	117 2017 786	25 1935 707	984 2054 759
200-299	106 2186 757	171 1996 640	89 2020 820	85 2013 592	58 1993 743	12 2037 795	521 2042 707
300+	164 2247 820	192 2051 729	89 2000 756	111 2186 828	79 2259 927	19 2027 772	654 2141 803
TOTAL	2552 2143 757	3033 2074 727	2162 2074 747	2449 2172 816	1616 2073 823	542 1988 768	12354 2104 771

each entry → n= number
mean absolute lymphocytes
standard deviation

TABLE II

ABSOLUTE LYMPHOCYTE COUNTS, FOURTH CYCLE EXAM (1964-66)

T65D EXPOSURE DOSE (rad)	AGE 0-29	30-39	40-49	50-59	60-69	70+	TOTAL
NIC	245 2094 672	288 2109 738	639 1972 659	612 2114 738	550 1990 703	225 1883 646	3059 2038 709
0-9	320 2061 620	1102 2071 701	774 1988 674	749 2139 761	717 2011 736	308 1864 715	3970 2040 712
10-49	169 2041 670	380 2109 683	359 2097 753	432 2114 741	412 1975 782	170 1810 639	1922 2047 732
50-99	52 1994 620	244 1979 640	198 2006 726	179 2042 627	196 1965 738	67 1724 725	936 1976 685
100-199	81 2014 600	350 2073 706	223 1939 696	186 2143 722	194 2025 912	71 1978 744	1105 2039 744
200-299	55 1928 596	203 2004 772	135 1937 661	107 2164 712	85 1958 712	37 2114 944	622 2011 730
300+	95 2130 782	216 2136 697	128 2084 761	110 2074 721	114 2215 696	44 2047 652	707 2123 721
TOTAL	1041 2050 654	3484 2074 709	2574 2004 692	2450 2123 740	2323 2005 752	937 1865 697	12809 2041 718

each entry → n= number / mean absolute lymphocytes / standard deviation

TABLE III

ABSOLUTE LYMPHOCYTE COUNTS, SEVENTH CYCLE EXAM (1970-72)

T65D EXPOSURE DOSE (rad)	AGE 0-29	30-39	40-49	50-59	60-69	70+	TOTAL
NIC	67 2167 775	322 2060 709	807 2064 704	499 2067 689	499 2010 701	354 1792 665	2548 2018 704
0-9	93 2077 650	448 2096 686	1013 2048 693	649 2095 729	674 1935 696	487 1758 618	3364 2000 697
10-49	39 1969 568	178 2085 740	386 2053 690	325 2136 739	377 1968 731	238 1739 642	1543 2003 717
50-99	10 1912 603	75 1981 594	288 2013 642	150 2088 657	160 1926 638	105 1689 590	788 1962 642
100-199	18 2004 620	89 2040 589	364 1968 681	189 2042 708	164 1888 638	111 1643 671	935 1938 677
200-299	12 1823 612	57 2094 902	213 1964 676	107 2149 889	83 1922 672	53 1779 649	525 1987 751
300+	36 2519 795	85 2316 916	225 2137 736	91 2048 768	93 2024 650	63 1835 612	593 2122 763
TOTAL	283 2114 698	1293 2087 719	3519 2042 694	2089 2094 727	2108 1959 690	1441 1757 637	10733 2005 704

each entry: n= number / mean absolute lymphocytes / standard deviation

TABLE IV

MEAN ABSOLUTE LYMPHOCYTE COUNTS TRANSFORMED BY THE SQUARE ROOT

Cycle #	AGE 0-69	70+	significance level
I	44.72	43.3	$\rho = .000075$
II	44.63	42.4	$\rho = < 10^{-16}$
III	44.52	41.2	$\rho = < 10^{-24}$

DISCUSSION:

A. Radiation exposure and absolute lymphocyte counts

Though no persisting effects of radiation on absolute lymphocyte counts was detected, a positive finding would not have been a surprise. Acutely, lymphocytes are exquisitely sensitive to radiation. Cronkite and Bond (50) have ranked mammalian tissue in order of decreasing susceptibility to radiation:

1. Spermatogonia
* 2. Lymphocytes
3. Erythroblasts
4. Remaining classical hematopoietic tissues
5. Epitheliumof small intestine
6. Stomach
7. Colon
8. Skin
9. CNS
10. Muscle
11. Bone
12. Collagen

With in vitro studies, Schrek (51) has demonstrated marked destruction of normal lymphocytes following exposure to as little as fifty rad of ionizing radiation. Following whole body irradiation, there is a rapid decline in lymphocytes from the peripheral blood, lymph nodes, thymus, spleen and other lymphoid tissues (20). This abrupt fall in lymphocyte population cannot be explained solely on the basis of damage to lymphocyte producing stem cells as even short lived lymphocytes have a relatively long intermitotic period (52). It is generally agreed that ionizing radiation has a "lympho-cytolytic" action similar to the action of pharmacological dose of corticosteroids.

of the atomic blasts in Hiroshima and Nagasaki had not been complete. The early findings were reported by Blaisdell (22) in a summary of hematological studies performed at the Atomic Bomb Casulaty Commission, in both cities, during the years 1947-59. In 1947-48 (19-32 months after exposure) the HE 67 survey of Hiroshima survivors was undertaken. The HE67 survey was a collection of 924 Hiroshima survivors, selected from greater than 16,000 people who had admitted on a questionaire to a history of epilation within 130 days of the atomic blast. At that time, whole body gamma radiation exposure was estimated to be 300-700 rad for this cohort (even though 20% of the individuals were more than 2000 meters from the hypocenter). The control sample consisted of 995 individuals from Kure city (approximately 50 km from Hiroshima). Leukocyte counts and white cell differentials were performed. Though the total leukocyte counts were approximately the same in both the exposed of Hiroshima and the Kure controls, the relative lymphocyte count was slightly lower and the eosinophil count slightly higher in Hiroshima.

In 1948-49, a followup HE 67 survey with a similar but not identical sample failed to demonstrate a difference in lymphocyte percentages between Hiroshima survivors and Kure controls.

During the years 1953-56 the ME 74 survey was conducted in Hiroshima. This sample was designed to focus on a population of heavily irradiated survivors. It consisted of 5000 adults who reported themselves to have been within a 2000 meter radius

of the hypocenter at the time of the blast and who gave a history of epilation, purpura or oropharyngeal ulceration. The "control" cohort was made up of a group matched for age and sex, who were reportedly 3000-3500 meters from hypocenter. There were no significant differences in lymphocyte percentages between exposed and "controls".

The results of the first five cycles of examination (1958-68) in the Adult Health Study Sample at ABCC were reported by Belsky et al (48). The effect of radiation on the lymphocyte counts were not analyzed at that time. The present study investigated this question for three isolated cycles.

In attempting to look retrospectively at absolute lymphocyte counts in the AHS, one must be aware of several confounding problems before trying to infer correlations with radiation exposure:

1) Blaisdell has noted that there was a fall in the mean leukocyte count in Hiroshima during the period of 1947-56 (9000/cc to 5500/cc). He points out that this decline was coincident with controls, and therefore not a radiation effect. White-cell differentials did not change significantly during the same period so that the absolute lymphocyte counts fell proportionately. A similar decline, during the same time, was noted in Nagasaki, albeit the absolute figures were slightly higher than in Hiroshima. As well, a general decline in leukocyte counts throughout Japan was suggested at that time. The decline in WBC is believed to be related to a reduction in intestinal parasitism and a shorter duration of bacterial

infections in the resident populations of Japan, coincident with improved hygiene standards and the introduction of antibiotics during this period. In reviewing white blood cell counts from the five cycles of examination, 1958-68, values appear to have stabilized.

2) It seemed probable that age at time of blast, as well as the vague phenomenon of "aging" might have an effect on absolute lymphocyte counts and needed to be controlled for if one was to look for an isolated radiation effect. The singular effects of "aging" on absolute lymphocyte counts will be discussed in the next section ("Aging" and the Lymphocyte) though a brief discussion of the possible potentiation of radiation effects by age will follow.

Experimental studies on rats and mice (58-59) have shown that the age of the animal at the time of irradiation plays a significant role in the development of "late" radiation effects (i.e. induction of neoplasms, lens opacities, life shortening..). The late effects were greater in those animals exposed at a young age, dropped off with exposure at increasingly older age groups, and often showed a second peak with exposure at a very old age. Jablon and Kato (7) , in studying the mortality of A-bomb survivors, reached a similar conclusion in regard to the leukemogenic effects of radiation. They reported that those most susceptible to this effect were either in the youngest or oldest age brackets at the time of exposure, while those in the intermediate age grouping (10-49) had a significantly lower risk.

The supposition that radiation could effect lymphocytes, years after exposure, is not without some precedence. Awa et al (12) has described chromosomal aberrations in circulating lymphocytes from atomic bomb survivors, twenty years after exposure. Though the isolated effects of radiation and "aging" might be too small to be detected, a potentiation, through the combination of the two, might be appreciated though it was not detected in the present study.

3) Lymphopenia is not unique to acute radiation exposure. Cassileth (60) has catalogued multiple causes of lymphopenia including immunoglubulin disorders (e.g. Wiskott-Aldrich Syndrome, thymic abnormalities), intestinal lymph loss (e.g. thoracic duct drainage, impaired intestinal lymph drainage as in Whipple's disease, severe right-sided heart failure), increased lymphocyte destruction (e.g. acute radiation exposure, chemotherapy, increased plasma corticoids) as well as a myriad of miscellaneous maladies including Hodgkin's disease, aplastic anemia, sarcoid, acute and chronic renal failure, terminal carcinoma and miliary tuberculosis. In order to isolate a pure radiation effect on absolute lymphocyte counts, the sample size must be large enough to render the relative effect of any of these other causes of lymphopenia (each with a small incidence) insignificant. It was felt that the sample size of approximately 20,000 people was large enough to provide this "washout" effect.

4) Absolute lymphocyte counts give no information concerning the relative population of B or T cells. It is conceivable that one

In animal studies, Hulse (53) described a bimodal disappearance of lymphocytes from the peripheral blood and bone marrow following sublethal irradiation. After a rapid decline in the majority of circulating lymphocytes, there was a slower drop off in the remaining population. This was understood to represent a initial "cytolytic" effect on mature lymphocytes followed by a slower decline due to failure of production by lymphopoietic cells.

In contrast to an isolated exposure to high doses of radiation, the effects of low dose, chronic radiation exposure (e.g. radiation workers, radiologists) on absolute lymphocyte counts have usually been towards lymphocytopenia, associated with a mild leukopenia (20). However, there have also been reports of leukocytosis with lymphocytosis (54-55).

The rate and extent of recovery from severe post-irradiation lymphocytopenia has not been well documented. Wald (20) has pointed out that the time intervals in the sequential depletion, reappearance, and complete reestablishment of lymphocyte populations has a variance with species as well as with the extent of exposure. In a study involving rabbits exposed to large doses of radiation (300 rad) lymphocyte recovery was incomplete at three months (56). Conard, in reporting on the Marshallese, a population exposed to fallout radiation, noted a relative lymphocyte deficit in the exposed cohort up to eight years after the accident (57).

Late studies on lymphocyte populations in the survivors

of these sub-populations could decrease with a compensatory increase in the other, yielding a normal absolute lymphocyte count. Data on B and T lymphocyte populations are not available from the AHS sample. Hopefully, in future years, with the implementation of simpler techniques for enumeration, these two populations of lymphocytes will be critically evaluated.

5) A cross-sectional approach (i.e. looking at an isolated cycle examination) is not ideal in that it lends itself to a selection bias. Subjects exposed at an old age might be the individuals who have age-related changes in their absolute lymphocyte counts. At the point in time where the cross sectional analysis is made, these individuals might no longer be living. A more ideal approach would be to follow any particular individual, longitudinally, through all examination cycles. This type of study will soon be undertaken at RERF.

B. Aging and the Lymphocyte

In the course of analyzing absolute lymphocyte counts for radiation effects, it was necessary to look at the isolated effect of age, at time of examination, in order that it might be controlled for when assessing radiation effects. As well, absolute lymphocyte counts in aging populations is a phenomenon with its own significance, independent of prior radiation exposure.

As the prototypical immunocyte, the lymphocyte is the focus of attention in the controversial immunologic theory of

aging; a theory proposed most notably by Walford (61). In essence, the theory purports that failure of the immune system leads to accelerated senescence by leaving the organism vulnerable to infection neoplasia and the so-called "auto-immune" diseases. This failure can come about either by failure of lymphocyte function or by simple reduction in the number of immunocytes. Lymphocyte function (e.g. response to allogeneic lymphocytes, poke-weed mitogens, and phyto-hemaglutinin, as well as delayed hypersensitivity reactions) has been shown to be impaired with increasing age (17,62-64) . The effects of age and/or "aging" on absolute lymphocyte counts, as well as B cell and T cell counts, have not been well documented. It is known that there is a continuous decline in the absolute lymphocyte count from infancy to puberty (approximately 5000/cc to 2000/cc). Betke (65) states that from puberty to advanced old age (90 years) no further changes occur. Weksler (64) reported indentical percentages of B and T cells in both a young (25-40) and old (75-96) age group. Shapleigh (66) as well, claims the absolute number of lymphocytes do not change as a function of age. Opposed to this is a recent publication (67) which compared a middle aged group of people with a cohort aged 65-86 and found a moderate lymphopenia in the older age group. Corosella (68) noted a significant fall in rosette forming T cells in a group aged 46-60. Conard (17) in his twenty year review of findings in the Marshallese, reports a very slight reduction in lymphocytes with increasing age in both the exposed and non-exposed groups.

Though Belsky, in his report on the first five examination cycles of the AHS at ABCC, states that the differential counts showed no age relationship, he also notes that the absolute lymphocyte counts were not examined. In the same section he states that, in general, white blood cell counts did decrease with age; the inference being that the absolute lymphocyte counts decreased proportionately.

In the present study, we were able to demonstrate a significant ($\rho<.0001$) drop of approximately 10% in absolute lymphocyte counts in the 70+ age group. Though this phenomenom has been suspected for aging populations throughout the world, this is the first study with a sample size large enough to convincingly demonstrate it. It is unclear whether the magnitude of this drop is sufficient to account for a reduction in immune surveillance to level where malignancies and autoimmune disorders become manifest.

Section 6

CONCLUSION:

This study demonstrates that absolute lymphocyte counts are not reliable markers of radiation injury except during the acute exposure period. A shift in the ratio of T and B lymphocytes without a reduction in the absolute number of lymphocytes was not ruled out.

An important finding unrelated to radiation exposure was the demonstration of a significant drop in absolute lymphocyte counts in a 70+ age population.

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